Barts and The London
School of Medicine and Dentistry

Oral Cancer Screening:

Targeting High-Risk South Asian Populations in the United Kingdom

Anand Lalli

Centre for Clinical and Diagnostic Oral Sciences

Institute of Dentistry

Barts and The London School of Medicine and Dentistry

This thesis is submitted to the University of London for the degree of Doctor of Philosophy

2012

Project Supervisors

Dr. Ahmad Waseem

Reader in Oral Biology
Centre for Clinical and Diagnostic Oral Sciences
Institute of Dentistry
Barts and The London School of Medicine and Dentistry

Prof. Farida Fortune

Professor of Medicine in Relation to Oral Health
Centre for Clinical and Diagnostic Oral Sciences
Institute of Dentistry
Barts and The London School of Medicine and Dentistry

Abstract

South Asians residing in the UK are known to be significantly different in terms of socio-economic and cultural influences from the UK population in general. They are at substantially higher risk of developing oral cancer (OSCC) and the potentially malignant disorder (PMD) oral submucous fibrosis (OSF). To overcome barriers to conventional health service use, a mobile dental unit was the base for screening within the South Asian community. Bilingual advocates ensured cultural acceptability and actively recruited high-risk individuals for screening as well as being involved at the secondary referral centre to facilitate attendance for definitive diagnosis of positive screened individuals.

In total 1596 high-risk individuals were screened and 5.4% referred with suspicious lesions. No OSCC was detected in any positive screened individuals but PMDs were confirmed in 29%, with dysplasia (15%) and OSF (9%) the commonest lesions referred. Due to the complex presentation of OSCC the most appropriate gold standard screening outcome is the detection of individuals who cannot be discharged from long-term follow-up at the secondary referral centre. On this basis screening specificity was 99% and Positive Predictive Value (PPV) 79%. The low PPV was attributed to the high prevalence of complex oral mucosal lesions (46%) that cannot be definitively diagnosed as benign by visual examination alone, which indicates diagnostic aids are required for screening this high-risk population.

Compliance with referral for positive screened individuals was only 76% and immediate incisional biopsy of positive screened individuals would be needed to improve this. In addition to histological detection of dysplasia, molecular markers of disease could readily be investigated by immunohistochemistry and the expression of keratins are ideal candidates due to their responsiveness to pathological signalling and abnormal expression in oral (pre)cancer. Analysis of 28 fresh frozen OSF samples and 6 site-matched controls, using a panel of 22 monoclonal antibodies, revealed changes in keratin 17 expression which correlated with disease severity.

A mobile dental unit staffed by suitably experienced dentists and cultural advocates and equipped for immediate histological diagnosis of positive screened individuals is required in order to undertake effective and ethical oral cancer screening in high-risk UK based South Asian populations.

Contents

List of Figures

CHAPTER ONE: Screening for Oral Cancer in South Asian Populations

List of Tables

Abbreviations and Symbols

aa	Amino Acid
BM	Basement Membrane
bp	Base Pair
CAG	Community Advisory Group
CI	Confidence Interval
CSQ	Cumulative Staining Quotient
HGNC	Human Genome Organisation Gene Nomenclature Committee
HSE	Health Survey for England
HTA	Health Technology Assessment Programme
H+E	Haematoxylin and Eosin
IARC	International Agency for Research on Cancer
IF	Intermediate Filament
ICER	Incremental Cost-Effectiveness Ratio
K	Keratin
KRT	Keratin gene
LP	Oral Lichen Planus
mAb	Monoclonal Antibody
MeSH	Medical Subjects Headings (in PubMed)
µ	micro
NHS	National Health Service
OR	Odds Ratio
OSF	Oral Submucous Fibrosis
OSCC	Oral Squamous Cell Carcinoma

PBS	Phosphate Buffered Saline
QALY	Quality-Adjusted Life-Years
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
TA cells	Transit-Amplifying cells
THPCT	Tower Hamlets Primary Care Trust
UK	United Kingdom
ULF	Unit Length Filament
v/v	volume/volume
WHO	World Health Organisation
w/v	weight/volume

Acknowledgements

One of the joys of completion is to look over the journey past and remember all the colleagues, friends and family who have helped and supported me on this long but fulfilling road.

First and foremost I would like to express my heartfelt gratitude to my supervisors Dr. Waseem and Prof. Fortune. I could not have asked for more inspirational role models and hope that I can in turn pass on the research values they have impressed on me. I am exceptionally grateful to Dr. Waseem for his time, guidance, immense patience and incredible efforts from day one and throughout this project and remain indebted to Prof. Fortune for allowing me to work in the Dental Institute and providing amazing support whenever it was needed.

I am also hugely fortunate to have worked with so many wonderful colleagues through the years and gratefully acknowledge those who have who have assisted with this project. Dr. Noha Seoudi, Dr. Stuart McEachen and Dr. John Buchanan from the Department of Oral Medicine, Barts and the London School of Medicine and Dentistry who managed the patients referred from the screening programme. Prof. Ray Croucher, Shamsia Begum and Dipul Ghosh from the Department of Dental Public Health, Barts and the London School of Medicine and Dentistry and Hazel Nunn, Jessica Harris, Zoë Norridge and Sara Hiom from Cancer Research UK who organised and funded development of the screening protocol. Prof. W. M. Tilakaratne and Dr. A. Ariyawardana from the University of Peradeniya, Sri Lanka for kindly providing the tissue samples used in immunohistochemical analysis. Prof. Irene M. Leigh from the College of Medicine, Dentistry and Nursing, University of Dundee who generously gifted the monoclonal antibodies used in immunohistochemical staining. I am also grateful to Dr. Ken White of London Metropolitan University and Dr Selvam Thavaraj of Kings College for their roles in the PhD examination committee.

I am privileged to have met and worked with so many incredible people in the Centre for Clinical and Diagnostic Oral Sciences and more recently Dr. Judith Jones and her team in the Oral Surgery department of the Institute of Dentistry.

Finally and forever more I thank my parents and family, accepting that any written expression of gratitude will never suffice for all that they have done. I keep in mind those who were there for as long as they could be, on this journey. You will never be forgotten.

Publications from this Thesis

An altered keratinocyte phenotype in oral submucous fibrosis: correlation of keratin K17 expression with disease severity.
Lalli A, Tilakaratne WM, Ariyawardana A, Fitchett C, Leigh IM, Hagi-Pavli E, Cruchley AT, Parkinson EK, Teh MT, Fortune F, Waseem A.
Journal of Oral Pathology and Medicine. 2008 Apr;37(4):211-20.

Oral cancer screening in the Bangladeshi community of Tower Hamlets: a social model.
Nunn H, Lalli A, Fortune F, Croucher R.
British Journal of Cancer. 2009 Dec 3;101 Suppl 2:S68-72.

Copies of each of the manuscripts are presented in the Appendix.

Declaration

Except for the assistance mentioned in the acknowledgements, the contents of this thesis are entirely my own work. This work has not been previously submitted, in part or in full, for a degree or diploma to this or any other University or Examining Board.

Signature…….. Date……...

Anand Lalli
10 Marlborough Road
Slough
Berkshire
SL3 7LH
a.lalli@qmul.ac.uk

CHAPTER ONE

SCREENING FOR ORAL CANCER IN SOUTH ASIAN POPULATIONS

Chapter 1

Screening for Oral Cancer in South Asian Populations

Oral cancer should be an ideal target for a screening because unlike the majority of other cancers, almost all oral cancers develop as visible lesions. Therefore, oral cancers should easily be detected early when treatment is simple, particularly because dentists are experts in detecting abnormalities of the oral cavity. Yet in 2012, oral cancer still presents a significant burden on the healthcare system and individuals still suffer immense morbidity from radical treatment and mortality rates still aren't improving because the majority of oral cancers are detected at a late stage.

1-1.01 What is Cancer?

Cancers or malignant neoplasms are a group of diseases characterised by unregulated cell growth. Cancer cells replicate and grow uncontrollably allowing them to invade adjacent tissues and spread to distant sites. The causes of cancer are multifactorial but usually result in damage to genes or actions in combination with existing genetic faults within cells. There are over 200 different cancers that affect humans, each with their own specific clinical, aetiological and pathological features [26]. Cancer is usually treated by destroying the malignant cells with cytotoxic drugs or radiation or removing them by surgical means. The chances of survival vary greatly by the type and location of the cancer and the extent of disease at the start of treatment. In 2007, cancer caused approximately 13% of all human deaths worldwide (7.9 million people) [27].

1-1.02 Definitions of Oral Cancer

There is no standard definition of 'oral cancer' in the literature. The broadest descriptions include any cancerous tissue located in the oral cavity which may arise as a primary lesion originating in any of the oral tissues or by metastasis from a distant site or by extension from neighbouring anatomic structures. Oral cancer as a sub-group of head and neck cancer is also often used synonymously with mouth cancer in the literature. Further confusion arises from the salivary gland neoplasms that maybe included in some definitions whilst others relate only to oral squamous cell carcinomas (OSCC), originating in the squamous epithelium that lines the mouth and lips. The definition used by organisations such as Cancer Research UK is "cancers of the lip, tongue, oral cavity, oropharynx, hypopharynx and piriform sinus" (www.cancerresearchuk.org). This definition has the advantage of directly relating to the World Health Organisation International Statistical Classification of Diseases and Related Health Problems (ICD) [28] codes ICD-10 C00 (lip), C01 (base of tongue), C02 (other and unspecified parts of the tongue), C03 (gum), C04 (floor of mouth), C05 (palate), C06 (other and unspecified parts of the mouth), C09 (tonsil) and C10 (oropharynx). These sites are also those that would be most accessible to direct visualisation on examination of the oral cavity and therefore most applicable to a screening programme.

1-1.03 The Clinical Presentation of Oral Cancer

In textbooks the lower lip is often cited as the most frequent site for OSCC around the oral cavity, due to direct actinic damage [8]. In the oral cavity the single most common site for lesions is the ventrolateral tongue and 70% of OSCCs develop in the 'gutter area' (Figure 1-01) formed by the ventrolateral border of the tongue, the

floor of mouth and the lingual mucosa, extending posteriorly towards the oropharynx. This is despite the ‘gutter area’ accounting for just 20% of the total mucosal surface area of the oral cavity and is likely to be due to carcinogens pooling and becoming concentrated in this area before being swallowed. For the same reason the dorsum of the tongue and hard palate are very rare sites for OSCC [8].

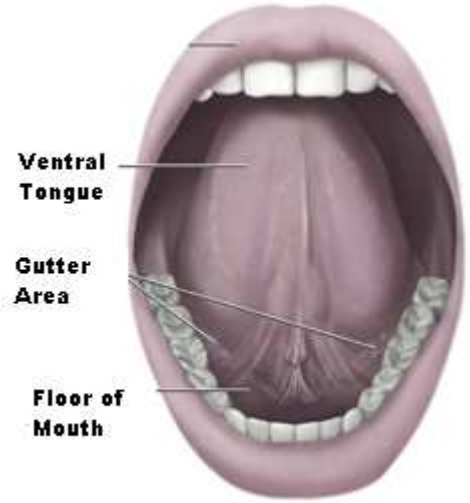


Figure 1-01: Conventionally the areas at highest risk of developing OSCC in the oral cavity are the ventral tongue, floor of mouth and lingual mucosa making up the ‘gutter area’.

A radically different pattern of OSCC development is seen in lesions where carcinogens are habitually placed against the mucosa. This is typical of areca nut extract usage where lesions appear at the site of placement of the areca nut compound and therefore most commonly occur on the buccal mucosa and in the lower buccal sulcus. This pattern of OSCC is particularly relevant to South Asian populations because of their inclination to areca nut extract usage.

Early OSCC lesions usually appear as painless red, speckled or white patches (Figure 1-02) with only a minority being ulcerated.

Figure 1-02: Early OSCC presenting as a mucosal white patch (Left - from *www.oralhealthnet.co.uk) and speckled patch (Right – from https://healthinessbox.wordpress.com)*

As the OSCC enlarges it may develop into a raised nodule or become ulcerated (Figure 1-03). The classical presentation of an indurated ulcer with rolled border indicates an advanced lesion. Pain is not a typical symptom used in the diagnosis of OSCC but ulceration can be associated with soreness or stinging when eating[8].

Figure 1-03: Advanced OSCC presenting as a raised nodular lesion (Left – from http://cancer-caregiver-treatment.com/Oral_Cancer.html) and an ulcerated lesion (Right – from http://www.deardoctor.com/articles/oral-cancer/page2.php).

1-1.04 Risk Factors for Oral Cancer

It has not been possible to prove conclusively a causative association between specific aetiological factors and OSCC, suggesting that the causes are multifactorial. Causative factors operate over a long period and malignant change must occur so slowly that there is a substantial lag period before it is clinically evident. The suggested risk factors for OSCC are: [8]

Carcinogens – tobacco, alcohol, areca nut.

Sunlight (lip lesions only).

Infections – syphilis, candida, viruses (e.g. HPV 16).

Mucosal disease – epithelial dysplasia, LP, OSF

Genetic disorders – dyskeratosis congenita, Fanconi's anaemia.

The effects of tobacco on the oral mucosa depend on the way it is consumed. In the UK cigarette smoking predominates and is believed to be the major aetiological factor for OSCC in synergistic combination with alcohol. Whereas, in South Asian countries tobacco chewing as well as smoking is a common habit and is frequently also associated with areca nut extract usage. Combinations of a quid of areca nut, lime, tobacco and spices wrapped in betel leaf as 'paan' (Figure 1-04) are held in the sulcus and this site is often where OSCC develops in these individuals. Areca nut releases the potent carcinogen arecolin which acts in addition to the multitude of carcinogens in tobacco. Areca nut extract usage is also the prime aetiological factor in the development of the potentially malignant disorder Oral Submucous Fibrosis (OSF).

Figure 1: *Leaf of betel* (Piper betle).

Figure 2: *Sliced areca nut, one of the major constituents of betel quid (paan), can also be chewed on its own.*

Figure 3: *Sweeteners are added to children's paan.*

Figure 4: *Once the ingredients have been placed on the betel leaf, the leaf is folded and the paan is ready to chew or suck.*

Figure 1-04: 'Paan' comprising areca nut quid, lime, tobacco and spices wrapped in betel leaf. (From: Avon (2004) [7])

1-1.05 Potentially Malignant Disorders of the Oral Cavity

Potentially malignant disorders (PMDs) are defined as "all clinical presentations that carry a risk of cancer" [15]. They are also variously referred to as precancer or premalignancy in the literature but the term PMD is preferred as it conveys that not all transform into cancer. The proportion of PMDs that change into oral cancer varies greatly, based on the characteristics of the disorder and its site in the oral cavity, the patient's age and gender [29], and the patient's behaviours [15].

Much of the literature on PMDs focuses on leukoplakia which is defined as "white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer" [15]. Leukoplakia is a clinical term with no

specific histological presentation and can present with or without the histological finding of epithelial dysplasia. Overall malignant transformation rate for leukoplakia is estimated at around 1% per year [30] or between 6.2 and 29.1 OSCCs per 100,000 population [31]. Some forms of non-homogenous leukoplakia are known to have a higher rate of transformation than clinically homogenous leukoplakia and these mixed white and red plaque like lesions are termed erythroleukoplakia. Erythroplakia is considered the PMD with greatest potential for malignant change and is defined as “a fiery red patch that cannot be characterized clinically or pathologically as any other definable disease.” [15] It is reported that between 75 and 90% of erythroplakia will undergo malignant transformation [32].

Dysplastic change is believed to be the best predictor of future malignancy in PMDs and the more severe the dysplasia the greater the likelihood of malignant transformation [33]. Barnes (2001) [34] suggests that the risk of developing OSCC in white patches with mild, moderate, and severe dysplasia is approximately 6%, 23%, and 28% respectively. Other estimates suggest that around 50% of all patients with dysplasia may go on to develop oral cancer [29]. Clinically leukoplakia and erythroplakia appear very similar to early OSCC (Figure 1-02) because of the fundamental underlying processes of epithelial keratinisation and dysplasia [8]. The natural history of OSCC is only partly understood but it is evident that OSCC is preceded by changes in the oral mucosa although the nature of these changes is still unclear. Nevertheless, it is believed that the majority of OSCCs are preceded by a detectable preclinical phase presenting as a PMD [29]. Management of these PMDs conventionally involves monitoring if not dysplastic or only mildly atypical. Dysplastic lesions are usually removed surgically or laser ablated although the

effect of this on risk of malignant change is not clear [35]. The suggestion is that a dysplastic lesion is merely a discrete manifestation of the fundamentally damaged mucosa affected by field change [36].

1-1.06 Oral Epithelial Dysplasia

Epithelial dysplasia is a disorder of differentiation of epithelial cells that may regress, remain stable or progress to carcinoma. The histomorphological changes indicative of keratinocyte maturation disturbances that are diagnostic findings in dysplasia, according to the World Health Organisation, are shown in Table 1-01:

Table 1-01: World Health Organisation histomorphological criteria for grading epithelial dysplasia. (From: Barnes et. al. (2006) [21])

Architecture criteria	Cytology criteria
1. Irregular epithelial stratification	1. Abnormal variation in nuclear size
2. Loss of polarity of basal cells	2. Abnormal variation in nuclear shape
3. Drop-shaped rete ridges	3. Abnormal variation in cell size
4. Increased number of mitotic figures	4. Abnormal variation in cell shape
5. Abnormally superficial mitoses	5. Increased nuclear–cytoplasmic ratio
6. Premature keratinisation in single cells	6. Increased nuclear size
7. Keratin pearls within rete ridges	7. Atypical mitotic figures
	8. Increased number and size of nucleoli
	9. Hyperchromatism

Dysplastic lesions are conventionally graded as ‘mild’, ‘moderate’ or ‘severe’ according to the extent of aggregate histomorphological abnormalities noted in tissue sections (Figure 1-05). However, the realisation that no truly reproducible criteria exist to precisely divide the spectrum of changes has resulted in proposed

simpler binary ‘low’ and ‘high’ risk classifications [37]. A diagnosis of carcinoma-in-situ is made when dysplastic features involve all surface epithelial strata (i.e. “top-to-bottom”). A diagnosis of SCC is made when there is evidence that nests of epithelial cells have invaded the underlying lamina propria and deeper submucosa.

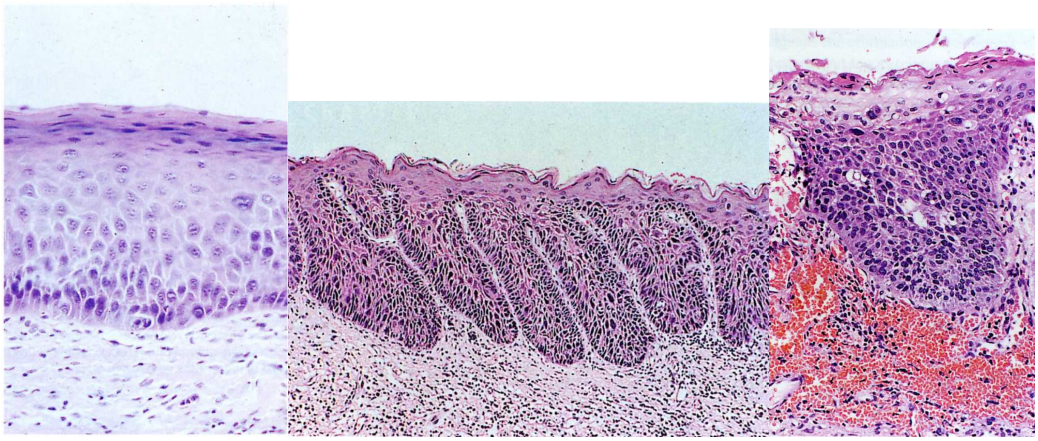


Figure 1-05: Histomorphological atypia in mild (left), moderate (centre) and severe (right) oral epithelial dysplasia.(From: Cawson and Odell (2002) [8])

1-1.07 Oral Submucous Fibrosis

Apart from dysplasia the transformation rate of other PMDs is harder to quantify, ranging from 0.13% to 2.2% for all PMDs combined [29]. Of specific relevance to South Asian populations, where areca nut extract usage is prevalent, is Oral Submucous Fibrosis (OSF). OSF was first described in the 1950’s as a chronic debilitating premalignant condition which affects millions of individuals worldwide [38]. Incidence varies between countries but it is most commonly seen on the Asian subcontinent and amongst migrants from these regions [9]. OSF studies frequently originate from countries such as India, Sri Lanka, Taiwan and China. Malignancy develops in 7-26% of OSF lesions [39] and these contribute to oral cancer being the 6th most common cancer worldwide [40].

1-1.08 OSF Aetiology

There is a direct relationship between the use of areca nut extract and development of OSCC in OSF, prompting the World Health Organisation to classify areca nut as a group I carcinogen [41]. Areca is a natural substance chewed for its psychostimulating effects. The nut of the areca palm (Areca catechu) is often combined with the leaf of the betel piper (Piper betle) and lime (calcium hydroxide). Additionally, tobacco products maybe added as well as other spices based on regional variations and customs. OSF pathogenesis is likely to be multi-factorial as only a small proportion of areca nut users actually suffer from this condition and consequently other genetic and nutritional factors have been implicated [39]. In India the number of OSF affected individuals is rising rapidly as a result of the relatively recent introduction of gutkha (paan masala) an areca-nut and tobacco mixture. Gutkha users have higher rates of OSF development than other areca preparations therefore it is viewed as especially ominous because of the youth appeal, the ease of procurement, low expense, convenient packaging, and the lack of social stigma [42].

1-1.09 OSF Clinical Presentation

Diagnosis of OSF is by its distinctive orofacial presentation in a patient with a history of areca nut use. OSF is characterised by progressive loss of elasticity of the oropharyngeal mucosa and atrophy of the oral epithelium and commonly results in restricted mouth opening, reduced tongue mobility and sensitivity to spicy food (Figure 1-06). Complications include conductive deafness from functional stenosis of the Eustachian tubes when the paratubal muscles are involved [43], dysphagia due to oesophageal involvement, vocal nasality and altered

salivary flow from salivary gland fibrosis. The associated morbidity includes nutritional deficiencies, as a result of a dietary limitations and rampant caries and periodontal disease, from the inability to maintain oral hygiene, as well as the psychological impact of a chronic progressive debilitating disorder with a significant risk of malignant conversion.

Figure 1-06: The clinical presentation of OSF in a 31 year old Indian woman with a 17 year history of guthka usage. Right and left buccal mucosa shows blanching, fibrous raphe and mucosal pigmentation combined with trismus and a shrunken uvula is characteristic of OSF. (From Reichart and Philipsen, 2006 [9])

1-1.10 OSF Clinical Classification

Pindborg (1989) [44] divided OSF progression into 3 phases based on the clinical presentation:

Stage 1: Stomatitis: including erythematous mucosa, vesicles, mucosal ulcers, melanotic mucosal pigmentation, and mucosal petechia.

Stage 2: Fibrosis occurring in ruptured vesicles and ulcers during healing:

- Early lesions demonstrate blanching of the oral mucosa.
- Older lesions include vertical and circular palpable fibrous bands in the buccal mucosa and around the mouth opening or lips.
- Specific findings include the following:

  Trismus

  Stiff and small tongue

  Blanched and leathery floor of the mouth.

  Fibrotic and depigmented gingiva

  Rubbery immobile soft palate

  Blanched and atrophic tonsils

  Shrunken uvula

  Sinking of the cheeks, not commensurate with age or nutritional status

Stage 3: Sequelae:

- dysplastic lesions and malignancy
- speech and hearing defects.

Khanna and Andrade (1995) [45] developed a classification of OSF associated trismus to aid clinical staging:

Group I:	The earliest stage and is not associated with mouth opening limitations (interincisal distance of >35 mm.)
Group II:	Interincisal distance of 26-35 mm.
Group III:	Moderately advanced cases. Interincisal distance of 15-26 mm with fibrotic bands visible in the soft palate, pterygomandibular raphe and anterior pillars of fauces.
Group IVA:	Advanced OSF with severe trismus (interincisal distance of less than 15 mm) and extensive fibrosis of all the oral mucosa.
Group IVB:	Severe trismus with interincisal opening of less than 15 mm, extensive fibrosis of the oral mucosa and (pre)malignant lesions.

1-1.11 OSF Management

If detected early OSF can be managed by education concerning the use of areca nut compounds and cessation of the habit is sufficient to prevent the disease progressing [39]. Unfortunately, most patients present when OSF is already severe and irreversibly limiting oral function. Treatment is then symptomatic and aimed at reducing further deterioration of mouth opening. A variety of topical and intralesional pharmacotherapeutic compounds (steroids, hyaluronidase [46], placental extracts [47], interferon γ [48] have been advocated but evidence of clinical effectiveness appears unreliable [49]. Mouth-opening physiotherapy exercises maybe just as helpful in preventing further limitation of mouth movements. Surgical management is indicated when severe trismus is prohibitive to feeding and communication. Surgery involves extensive split-thickness skin grafting or nasiolabial or lingual pedicle flaps as simple excision of the fibrous bands often results in contracture and exacerbation of the condition. Surgical management is

further complicated by the difficulties of laryngoscopy and intubation of the trachea [50]. The potential healthcare burden of OSF is hugely increased by late detection and the substantial risk of malignant transformation necessitating frequent biopsy and long term follow-up. Therefore, as with OSCC, there are substantial advantages to the early detection of OSF.

1-1.12 Referral Guidelines for Suspected Oral Cancer

OSCC develops in the superficial epithelium lining the oral cavity and is therefore relatively open to visual assessment although histological examination is the gold standard required to confirm the diagnosis. The National Institute of Health and Clinical Excellence (NICE) has issued referral guidelines for suspected cancer [10] to be used by primary care practitioners (e.g. general dental and medical practitioners). These guidelines use 3 referral timelines:

- **Immediate**: an acute admission or referral occurring within a few hours.
- **Urgent**: the patient is seen within the national target for urgent referrals (currently 2 weeks).
- **Non-urgent**: all other referrals.

Oral cancer is included in this document under “head and neck cancer including thyroid” but specific guidance for OSCC is given (Figure 1-07):

1.11.4 In a patient who presents with unexplained red and white patches (including suspected lichen planus) of the oral mucosa that are:

- painful, or
- swollen, or
- bleeding

an urgent referral should be made.

A non-urgent referral should be made in the absence of these features. If oral lichen planus is confirmed, the patient should be monitored for oral cancer as part of routine dental examination.[9] **C**

1.11.5 In patients with unexplained ulceration of the oral mucosa or mass persisting for more than 3 weeks, an urgent referral should be made. **C**

1.11.6 In adult patients with unexplained tooth mobility persisting for more than 3 weeks, an urgent referral to a dentist should be made. **C**

1.11.8 In patients with an unexplained lump in the neck which has recently appeared or a lump which has not been diagnosed before that has changed over a period of 3 to 6 weeks, an urgent referral should be made. **C**

Figure 1-07: Descriptions of OSCC from the NICE Referral Guidelines for Suspected Cancer (2005).[10]

These guidelines are quoted as “Recommendation Grade C” or “directly based on category III evidence or extrapolated recommendation from category I or II evidence” [10] (Table 1-02). This suggests that the evidence for these recommendations is either from well-designed non-experimental descriptive studies or case-control studies or case series. Alternatively, they may be extrapolated from randomised controlled trials (RCTs) or systematic reviews of RCTs [22], indicating the dearth of high quality evidence available relating to the referral of OSCC.

Table 1-02: Levels of evidence applied to NICE Referral Guidelines for Suspected Cancer. (Adapted from Eccles and Mason (2001) [22])

Evidence category	Source
Ia	Evidence from systematic review or meta-analysis of randomised controlled trials
Ib	Evidence from at least one randomised controlled trial
IIa	Evidence from at least one well-designed controlled study without randomisation
IIb	Evidence from at least one well-designed quasi-experimental study, such as a cohort study
III	Evidence from well-designed non-experimental descriptive studies, case-control studies, or case series
IV	Evidence from expert committee reports, opinions and/or clinical experience of respected authorities

The NICE Referral Guidelines on Suspected Cancer (2005) [10] additionally provide guidance on the role of primary care practitioners in investigation of OSCC which is “investigations for head and neck cancer in primary care are not recommended as they can delay referral.” Therefore the role of the dentist in diagnosis of OSCC is limited to examining patients and referring any suspect lesions i.e. essentially screening patients for OSCC.

These guidelines also provide relevant guidance for the management of patients from different cultures. “Primary healthcare professionals should provide culturally appropriate care, recognising the potential for different cultural meanings associated with the possibility of cancer, the relative importance of family decision-making and possible unfamiliarity with the concept of support outside the family.” [10] This would suggest that patients ideally need culturally appropriate advocates to interpret cancer related information whenever the referring practitioner is not of the same cultural background as the patient.

1-1.13 The Epidemiology of Oral Cancer in the UK

Cancer registration databases are held by 8 regional Cancer Registries in England along with registries in Wales, Scotland and Northern Ireland. Directly collated data is presented by the National Cancer Intelligence Network (NCIN) [http://www.ncin.org.uk]. Additionally for England the governments Office for National Statistics (ONS) [http://www.ons.gov.uk] provides data from the regional cancer registries in its yearly report. Cancer Research UK presents this data for the UK on their website [http://www.cancerresearchuk.org/cancer-info/cancerstats/types/oral]. The latest data shows that in 2009, 6,236 people were diagnosed with OSCC making up 2% of all cancers diagnosed in the UK. Therefore OSCC is the UK's 15th most common cancer and in 2010 directly resulted in 1,985 deaths with the lifetime risk of developing OSCC in the UK estimated to be 1 in 93 for men and 1 in 186 for women.

1-1.14 Oral Cancer and Geography in the UK

The incidence rates for OSCC in the UK in 2009 show that there are more than 10 new oral cancer cases for every 100,000 people in the UK (Table 1-03).

Table 1-03: Oral Cancer (C00-C06,C09-C10,C12-C14): 2009. Number of New Cases, and Incidence Rates per 100,000 Population Countries of the UK (Adapted from www.cancerresearchuk.org/cancer-info/cancerstats/types/oral/incidence)

		England	Wales	Scotland	Northern Ireland	UK
Male	**Cases**	**3,246**	**236**	**501**	**114**	**4,097**
	Incidence Rate	12.7	16.1	19.9	13	13.5
Female	**Cases**	**1,689**	**121**	**270**	**59**	**2,139**
	Incidence Rate	6.4	7.9	10.1	6.5	6.8
Persons	**Cases**	**4,935**	**357**	**771**	**173**	**6,236**
	Incidence Rate	9.5	11.9	14.8	9.7	10.1

The geographical variation in incidence across the UK reflects the prevalence of the two most well established risk factors which are excessive alcohol consumption and smoking. This North-South divide in oral cancer incidence (particularly for males) has existed across the UK since at least the 1990s [12].

1-1.15 Oral Cancer and Age

Oral cancer incidence in the UK is strongly related to age. For men, age-specific incidence rates increase sharply from the mid-forties and peak at ages 60-69, before falling in the over 70s. Age-specific incidence rates increase much more gradually for women, from around age 45, but peaking in the over-80s. (Figure 1-08)

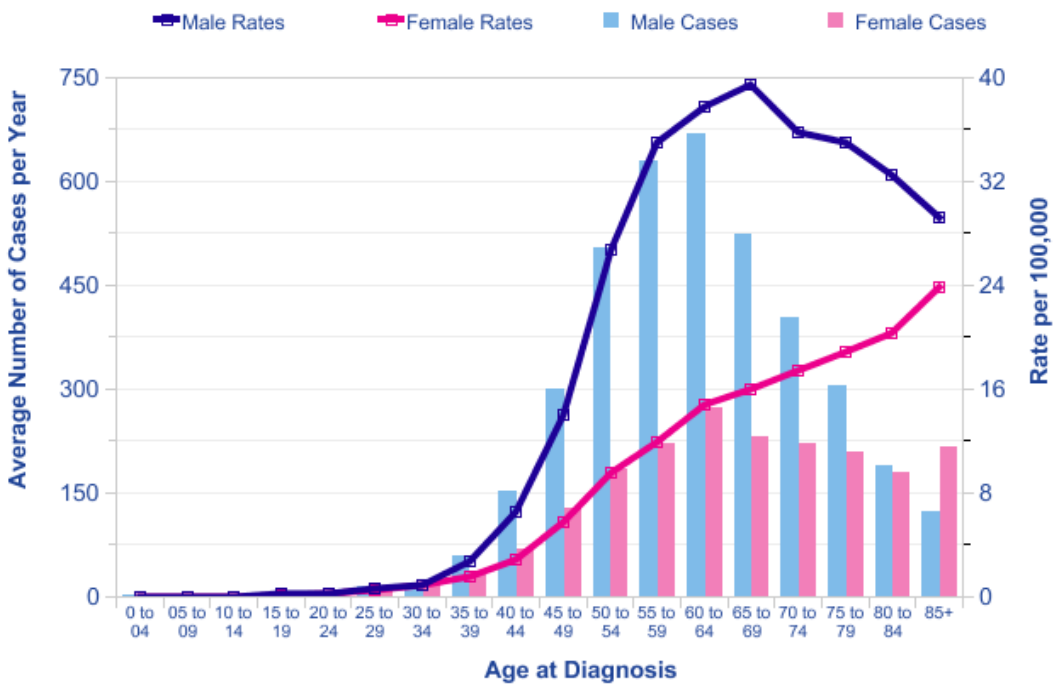


Figure 1-08: Oral Cancer (C00-C06,C09-C10,C12-C14): 2007-2009. Average Number of New Cases Per Year and Age-Specific Incidence Rates per 100,000 Population, UK. (www.cancerresearchuk.org/cancer-info/cancerstats/types/oral/incidence)

1-1.16 Oral Cancer Trends over Time

The incidence rates for all cancers increased by 20% in males during the period from 1975-2009 and by 40% in females with almost this entire rise occurring before the late 1990s. Over the last 10 years (1998-2009), the incidence rates increased by just 3% and 5%, respectively. (Figure 1-09).

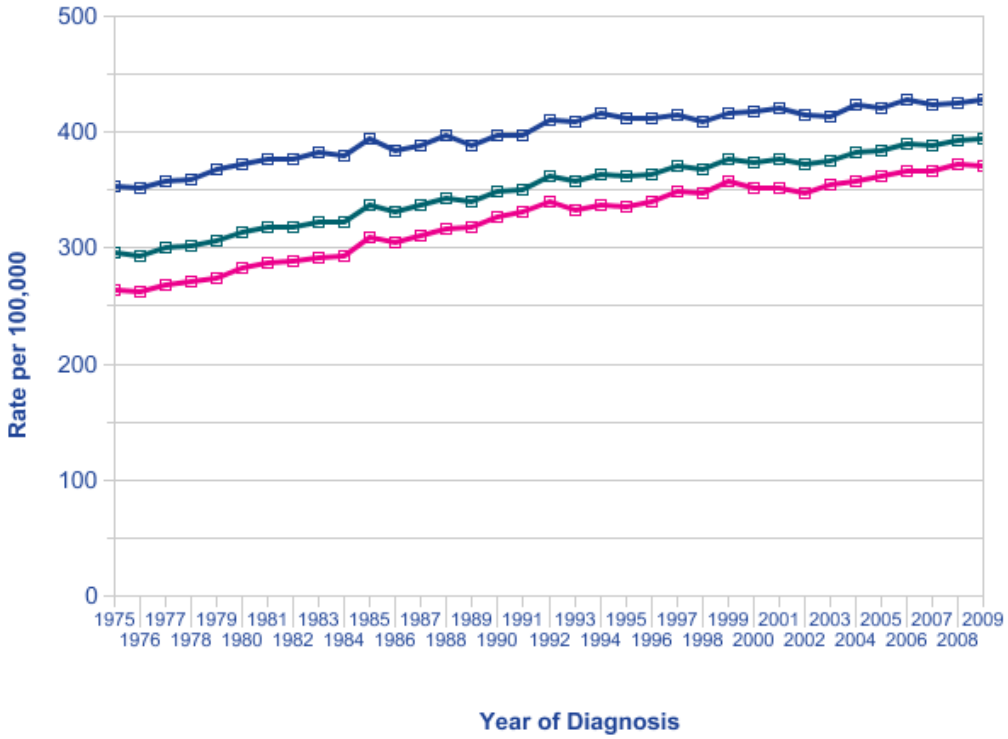


Figure 1-09: All Cancers Excluding Non-Melanoma Skin Cancer (C00-97 excl. C44): 1975-2009. European Age-Standardised Incidence Rates per 100,000 Population, by Sex, Great Britain. (www.cancerresearchuk.org/cancer-info/cancerstats/types/oral/incidence)

As with all cancers in total, OSCC incidence rates have increased since the mid-1970s. However, a large proportion of the increase has occurred since the late 1980s (Figure 1-10). Immigrants from the Indian sub-continent, may have partly contributed to the rising trend [51]. This also suggests that OSCC will become a more significant cancer issue in the UK in the future.

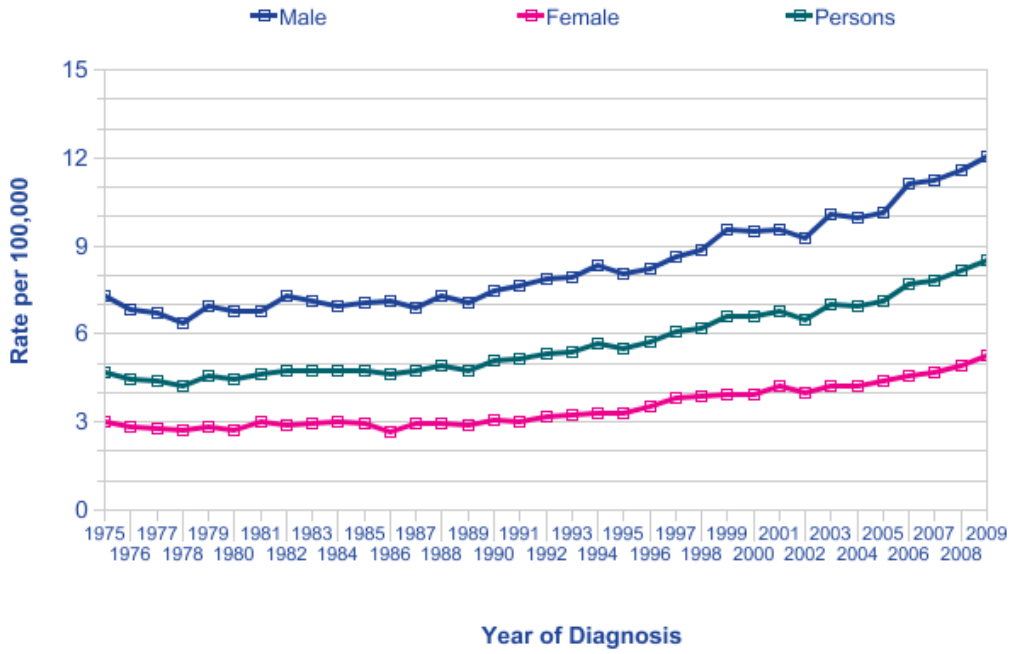


Figure 1-10: Oral Cancer (C00-C06,C09-C10,C12-C14): 1975-2009. European Age-Standardised Incidence Rates per 100,000 Population, by Sex, Great Britain. (www.cancerresearchuk.org/cancer-info/cancerstats/types/oral/incidence)

1-1.17 Oral Cancer Incidence Worldwide

Cancer registration systems are available in affluent countries such as the UK but 80% of the world's populations live in regions that are not covered by such systems [52]. Therefore, the International Agency for Research on Cancer (IARC) attempted to use all available data to estimate worldwide cancer incidence in the 2008 GLOBOCAN project [http://globocan.iarc.fr]. An estimated 400,000 new cases of cancer of the lip and oral cavity (ICD-10 C00-C08) and pharynx excluding the nasopharynx (C09-C10, C12-14) were diagnosed across the world in 2008, which is about 3% of all cancers Worldwide [52].

For lip and oral cavity cancer (ICD-10 C00-C08) the incidence rates are highest in South-Central Asia (Figure 1-11). Much of the geographical variation in incidence

can be attributed to differences in tobacco smoking and alcohol consumption, whilst areca nut extract usage is an important risk factor for some South Asian and Chinese populations. In high-risk countries such as Sri Lanka, India, Pakistan and Bangladesh, cancer of the lip and oral cavity is either the most common or second most common cancer in men, accounting for up to 15% of all new cases of cancer in males [52].

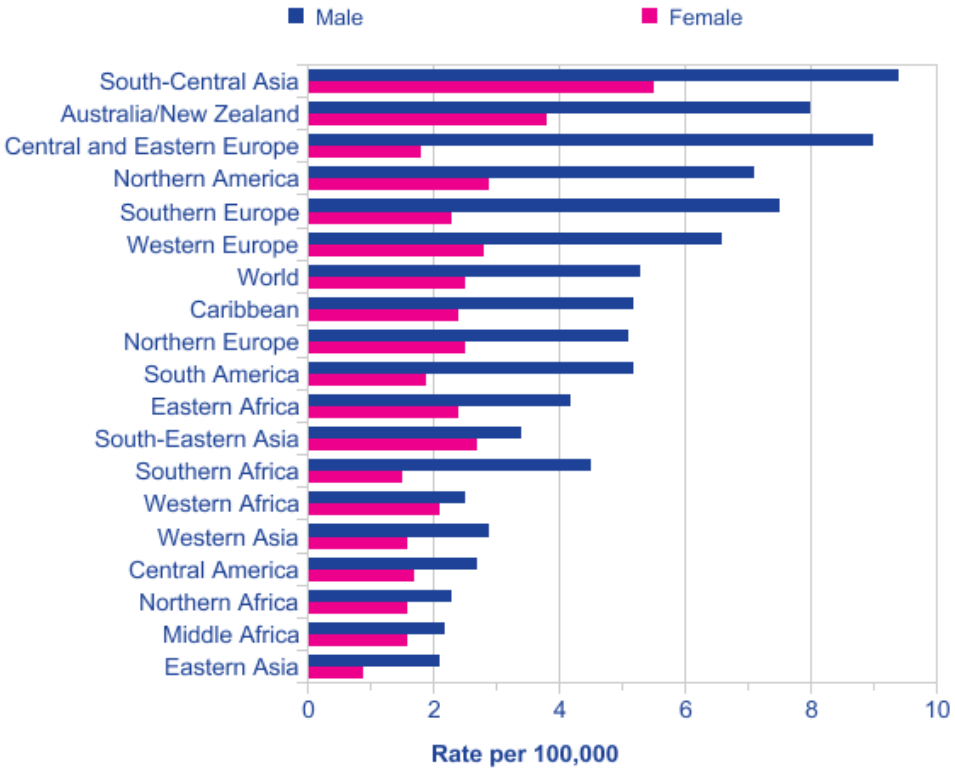


Figure 1-11: Lip and Oral Cavity Cancer (C00-C08): 2008 Estimates. World Age-Standardised Incidence Rates per 100,000 Population, World Regions. (www.cancerresearchuk.org/cancer-info/cancerstats/types/oral/incidence)

1-1.18 Mouth Cancer and Ethnicity

Incidence of all cancers as a whole is substantially lower in Asian populations in the UK compared to the White population as shown in Figure 1-12, even when worst case scenario assumptions have been made for the large number of cases of unknown ethnicity in the data set [11].

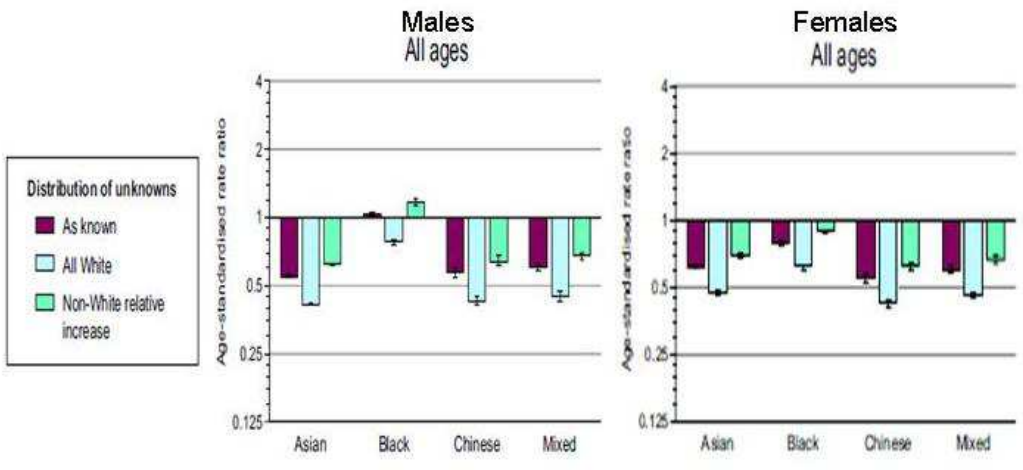
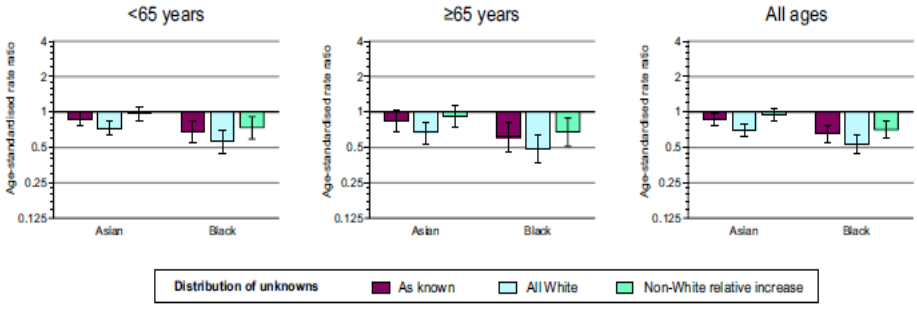


Figure 1-12: Rate Ratios for All Malignant Neoplasms Excluding Non-Melanoma Skin Cancer (C00-C97 excl. C44) (with 95% confidence intervals) by major ethnic group (White ethnic group = 1), Males and Females, UK. (Adapted from: Cancer Incidence and Survival by Major Ethnic Group, England. 2002-2006. National Cancer Intelligence Network 2009 [11])

The same NCIN report [11] reveals a very different distribution for mouth cancer amongst Asian populations in the UK (Figure 1-13). Results have only been presented for White, Asian and Black ethnic groups due to the small number of patients for the other ethnic groups and again there is a large proportion of unknown ethnicity (approximately 20%) for which a number of statistical scenarios have been calculated. However, the contrast with the data for all cancers is clear, as Asian women in the UK have significantly higher incidences of mouth cancer than the White population. The picture is less clear for Asian males because the apparent differences shown in Figure 1-13 were not statistically significant. Moles et. al. 2008 [53] confirmed this with data from the Thames Cancer Registry showing that after controlling for socioeconomic deprivation, South Asian males showed a marginally higher relative risk of oral cancer (OR 1.36; 95% CI: 1.11-1.67) than non-South Asian males. Whilst, South Asian females had a much higher risk of these cancers (OR 3.67; 95% CI: 2.97-4.53) than non-South Asian females. This

suggests that UK Asians are significantly different from the UK population as a whole in their risk for oral cancer.

MALES



FEMALES

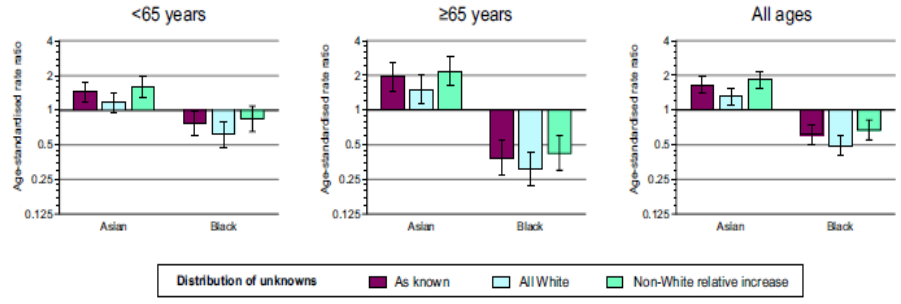


Figure 1-13: Rate Ratios for Mouth Cancer (C00-C08) *(with 95% confidence intervals) by major ethnic group (White ethnic group = 1), Males and Females, UK. (Adapted from: Cancer Incidence and Survival by Major Ethnic Group, England. 2002-2006. National Cancer Intelligence Network 2009 [11])*

1-1.19 Head and Neck Cancer and Social Deprivation

NCIN report that head and neck cancer shows a strong association with social deprivation (Figure 1-14) such that the incidence rates in the most deprived quintile of the population were 2.1 times that in the most affluent quintile [12]. It is also known that risk factors for cancer, especially smoking, are strongly influenced by socio-economic determinants [12]. Additionally, within London exist some of the most deprived boroughs in the UK, such as Tower Hamlets and Newham, which also have the highest proportion of Asian immigrant populations [54].

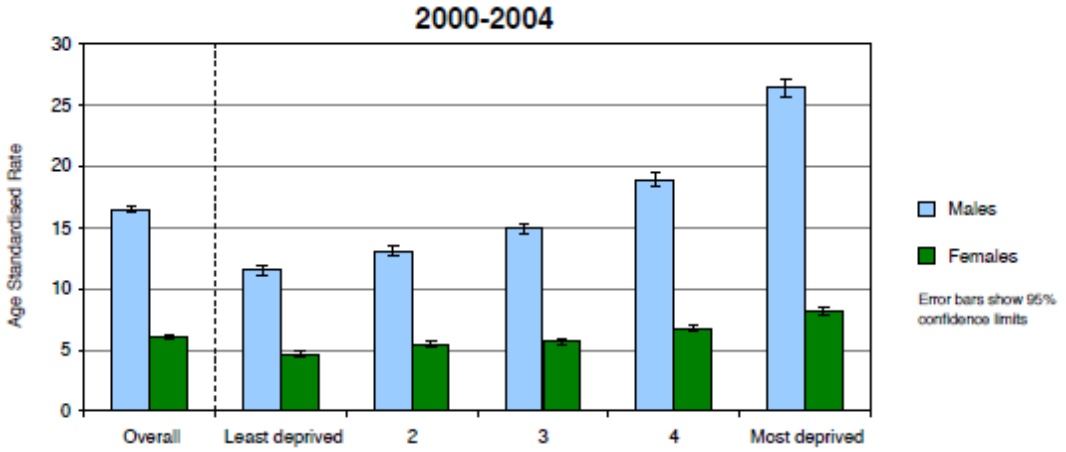


Figure 1-14: Cancer incidence by deprivation quintile, England, 2000 – 2004. (Adapted from: Cancer Incidence by Deprivation, England. 1995-2004. National Cancer Intelligence Network 2008 [12])

1-1.20 The TNM Classification of Oral Cancer

The TNM classification is the globally accepted method of describing the anatomical extent of any cancer. The OSCC specific TNM classification is shown below [55]:

T — Primary tumour

TNM	
TX	Primary tumour cannot be assessed
T0	No evidence of primary tumour
Tis	Carcinoma in situ
T1	Tumour 2 cm or less in greatest dimension
T2	Tumour more than 2 cm but not more than 4 cm in greatest dimension
T3	Tumour more than 4 cm in greatest dimension
T4a (lip)	Tumour invades through cortical bone, inferior alveolar nerve, floor of mouth, or skin (chin or nose)
T4a (oral cavity)	Tumour invades through cortical bone, into deep/extrinsic muscle of tongue (genioglossus, hyoglossus, palatoglossus, and styloglossus), maxillary sinus, or skin of face
T4b (lip and oral cavity)	Tumour invades masticator space, pterygoid plates, or skull base; or encases internal carotid artery

Note: Superficial erosion alone of bone/tooth socket by gingival primary is not sufficient to classify a tumour as T4.

N - Regional Lymph Nodes (Cervical)

NX	Regional lymph nodes cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in a single ipsilateral lymph node, 3 cm or less in greatest dimension
N2	Metastasis as specified in N2a, 2b, 2c below
N2a	Metastasis in a single ipsilateral lymph node, more than 3 cm but not more than 6 cm in greatest dimension
N2b	Metastasis in multiple ipsilateral lymph nodes, none more than 6 cm in greatest dimension
N2c	Metastasis in bilateral or contralateral lymph nodes, none more than 6 cm in greatest dimension
N3	Metastasis in a lymph node more than 6 cm in greatest dimension

Note: Midline nodes are considered ipsilateral nodes.

M – Distant metastasis

MX	Distant metastasis cannot be assessed
M0	No distant metastasis
M1	Distant metastasis

1-1.21 Clinical Staging and Prognosis

The TNM classification also allows clinicians to stage OSCCs which can then be correlated with survival outcomes.

Stage Grouping

Stage 0 (precancer)	Tis	N0	M0
Stage I (early)	T1	N0	M0
Stage II (locally advanced)	T2	N0	M0
Stage III (spread to lymph)	T1, T2	N1	M0
	T3	N0, N1	M0
Stage IVA (metastatic)	T1, T2, T3	N2	M0
	T4a	N0, N1, N2	M0
Stage IVB (metastatic)	Any T	N3	M0
	T4b	Any N	M0
Stage IVC (metastastic)	Any T	Any N	M1

Two year survival statistics (Table 1-04) reveal that small early OSCCs (stage I) without lymphatic spread have a substantially better prognosis than more advanced disease at diagnosis. Metastatic spread either to the regional lymph nodes or distant (stage III or IV) worsens the prognosis still further. Therefore early detection of OSCC is fundamental for improving outcomes as greater than 60% of OSCC are currently stage III or IV on presentation [56].

Table 1-04: Staging at diagnosis and two-year survival for cancers of the oral cavity, South West of England 1996-2000. *(Adapted from: www.cancerresearchuk.org/cancer-info/cancerstats/types/oral/incidence)*

	Oral cavity (% of cases)	2-year Survival (%)
All cases		62.0 (57.3-66.7)
I Early disease	21	87.5 (80.6-94.4)
II Locally advanced	17	68.6 (57.6-79.6)
III Tumour in lymph nodes	15	52.5 (40.0-65.0)
IV Metastatic	36	46.0 (38.0-54.0)
Unknown	11	68.2 (54.5-81.9)

1-1.22 Management of Oral Cancer

OSCCs are staged at diagnosis to give an indication of prognosis as well as treatment strategies. Standard treatment usually involves surgical resection of the tumour with a wide margin. Neck dissection may be required to remove associated lymph nodes as well as flap reconstruction of the defect [57]. Radiotherapy is conventionally used in combination with surgery when clear excision margins are not possible due to the extent of the tumour resulting in unacceptable disfigurement or debilitation, if excised completely [58]. Some OSCCs may also be untreatable, such as stage IV lesions with distant metastases, where management would be limited to palliation. Therefore, earlier diagnosis of smaller less-advanced OSCCs is beneficial in terms of morbidity as well as mortality as late detection necessitates more invasive treatment producing significant facial deformity and difficulty in eating, speaking, taste and the increased risk of frequent or rapidly progressing infections.

1-1.23 Screening and Case-Finding

Screening for disease implies an ongoing, structured healthcare intervention designed to detect disease at an asymptomatic stage when its natural course can be readily interrupted if not cured. 'Screening' is defined as the application of a test to people who are apparently free from disease in order to determine those who might have the disease from those who probably do not. In contrast, 'case-finding' is defined as a diagnostic test that is applied to a patient who has abnormal signs or symptoms in order to establish a diagnosis and aid treatment planning. In the literature, screening (detection) and case-finding (diagnosis) are used interchangeably.

1-1.24 The UK National Screening Committee

Because of the potential for over-diagnosis (false positives) and associated cost implications the UK National Screening Committee lists 22 criteria relating to both the condition and the screening test that should be met before a screening programme is introduced [59].

The Condition

1. The condition should be an important health problem

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage.

3. All the cost-effective primary prevention interventions should have been implemented as far as practicable.

4. If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.

The Test

5. There should be a simple, safe, precise and validated screening test.

6. The distribution of test values in the target population should be known and a suitable

cut-off level defined and agreed.

7. The test should be acceptable to the population.

8. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals.

9. If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out.

The Treatment

10. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment.

11. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered.

12. Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme.

The Screening Programme

13. There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an "informed choice" (e.g. Down's syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened.

14. There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public.

15. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment).

16. The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (i.e. value for money). Assessment against this criteria should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource.

17. All other options for managing the condition should have been considered (e.g. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources

available.

18. There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards.

19. Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme.

20. Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice.

21. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public.

22. If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members.

In 2010 Speight and Warnakulasuriya [60] attempted to address these criteria for oral cancer screening. The major issues were:

Criteria 2 - The natural history of OSCC is only party understood as PMDs exist but their progression to OSCC is unclear.

Criteria 6 - For visual examination of the oral tissues the test values in the target population are unknown so an appropriate test cut-off value is unclear for when to refer detected lesions or even when a lesion is present. Dentists use a variety of clinical examination findings to make their diagnosis but the decision to refer occurs only for lesions presenting as advanced OSCC [61].

Criteria 15 - There is no data on potential psychological harm from false positives in oral cancer screening.

They concluded that “given these substantial obstacles, it seems that screening cannot at the present time be advocated.” Although they accepted that “there is a

deep feeling within the profession that there should be some sort of screening programme for oral cancer." [60]

1-1.25 Sensitivity and Specificity

Sensitivity and specificity are statistical measures of the performance of a binary classification test e.g. the outcomes are either screen positive or negative.

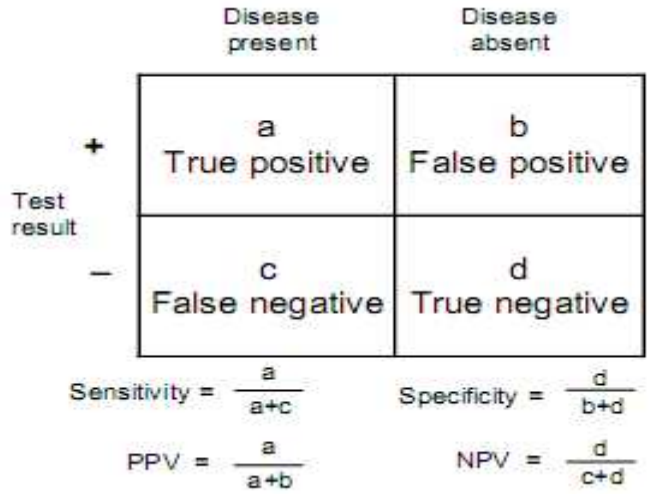


Figure 1-15: The standard 2x2 table for calculation of sensitivity and specificity of a screening programme. (Adapted from Lingen et. al. 2008 [13])

Sensitivity measures the proportion of subjects with the disease who test positive e.g. the percentage of people with OSCC who are screened positive.

$$\text{sensitivity} = \frac{\text{number of true positives}}{\text{number of true positives} + \text{number of false negatives}}$$

The specificity determines the proportion without the disease who test negative e.g. the percentage of healthy people who are screened negative.

$$\text{specificity} = \frac{\text{number of true negatives}}{\text{number of true negatives} + \text{number of false positives}}$$

For any test, there is usually a trade-off between sensitivity and specificity e.g. in an OSCC screening programme the dentists may screen positive non-specific PMDs (low specificity) in order to reduce the risk of missing an OSCC (high sensitivity). Furthermore, sensitivity and specificity must be considered together to evaluate a screening programme as theoretically any dentist who screened positive every participant in the trial would have 100% sensitivity (as all OSCCs would have been screened positive) but specificity would be abysmal as all healthy participants were not screened negative.

The positive predictive value (PPV) determines the proportion of subjects with positive screening results that do have the disease.

$$PPV = \frac{\text{number of True Positives}}{\text{number of True Positives} + \text{number of False Positives}} = \frac{\text{number of True Positives}}{\text{number of positive calls}}$$

The negative predictive value (NPV) gives the proportion of negative screening results that do not have the disease.

$$NPV = \frac{\text{number of True Negatives}}{\text{number of True Negatives} + \text{number of False Negatives}} = \frac{\text{number of True Negatives}}{\text{number of Negative calls}}$$

There are no defined values for the ideal screening test so it is desirable to have both high specificity (few false positives) and high sensitivity (few false negatives). The acceptable trade-off between sensitivity and specificity depends on the consequences of failing to detect the disease versus the costs, anxieties and other associated burdens from false positive results.

Studies have shown that oral cancer can be detected by direct visual examination of the oral mucosa with overall sensitivity 85%, specificity 97%, PPV 70% and NPV 98% [62]. In contrast, visual screening for skin lesions such as melanoma, has shown much higher sensitivity (93%) [63] suggesting that the heterogeneity of early OSCC and particularly PMD clinical presentations hampers detection. For this reason OSCC screening by direct visual examination will always result in a low PPV, indicative that many of the screen positive results are false positives. Therefore, it will be necessary to follow up any screen positive result with a more reliable test, such as scalpel biopsy and histological assessment, to obtain a more accurate assessment as to whether OSCC is actually present. Nevertheless, a low PPV screening test, such as direct visual assessment for OSCC, can be useful because it is inexpensive and convenient. The strength of direct visual assessment for OSCC as a screening test is in its high NPV, indicating that if an individual is screened negative, there is a high level of confidence that the individual is healthy.

When interpreting sensitivity and specificity calculations the prevalence of the disease is important, because if the disease is rare, such as OSCC, then even a very high sensitivity and specificity of the screening test will result in a high proportion of false positive outcomes. We can conclude that visual screening for OSCC, even in a high-risk population, will inevitably result in many false positives, the potential significance of which has not been investigated [60].

1-1.26 Cost-Effectiveness of Screening for Oral Cancer

A recent report by the NHS Health Technology Assessment Programme (HTA) attempted to assess the cost effectiveness of screening for OSCC in UK primary care services [64]. Eight screening methodologies were modelled in primary care settings as a one-off screening of a hypothetical population, over 40 years of age, followed for 60 years. The main outcome measures were mean lifetime costs and quality-adjusted life-years (QALYs) of each alternative screening scenario and incremental cost-effectiveness ratios (ICERs) to determine the additional costs or benefits of one strategy over another. The authors state that the targeted 'high-risk' screening strategies they modelled, utilise an approach in which high-risk patients are identified based on known risk factors of demographic and lifestyle characteristics (e.g. age, gender, smoking and alcohol) [64, 65]. These are risk factors for the UK population as a whole and may exclude the South Asian origin sub-population because their main risk factor for OSCC (areca nut extract usage) is not included in the modelling system.

1-1.27 Modelling Data from an 'Expert Panel'

In the HTA report [64] a complex set of statistical models were meticulously presented for each scenario but inevitably much of the data utilised information gained from an 'expert panel' on those areas for which there is no suitable data in the literature. An appropriate example of this would be the calculation of cost of oral cancer detection in primary care. Sixteen GDPs, who had recently been involved in a pilot oral cancer screening programme in their own dental practices, were sent questionnaires asking questions related to the time taken for a basic NHS examination and the extra time needed to carry out and record a thorough

examination of the oral soft tissues. Expert opinion was also sought from a number of experienced GDPs and hospital specialists. The actual costs were determined from published fee scales for GDPs. The costs allowed only for the additional time the dentist would need to examine the soft tissues and record the information. No costs were allowed for additional consumables, since it was assumed that the mucosal examination would be carried out as a part of a routine dental examination. Unfortunately, there is no indication why only 4 of 16 GDPs returned completed questionnaires but the mean extra time required to carry out a mucosal examination was 2.63 minutes. Expert opinion suggested that this was a reasonable time and was equivalent to the extra time allowed within the General Dental Services for an ‘extended examination’. The cost difference between a routine examination and an extended examination was £3.40 therefore this was used as an approximation of the cost of an OSCC mucosal examination. Clearly, there is no way of quantifying the accuracy of this approximation to take into account other aspects of an extended examination such as periodontal probing, or the accuracy of the dentist’s mucosal examination. As this cost is fundamental to undertake any modelling exercise of cost effectiveness, generic inaccuracies and approximations must be assumed to be accounted for by statistical error calculations such as the probability distributions assigned to each input parameter in the model. The author’s discussion states “Despite the low response rate, the responses from the GDPs were only used to check the robustness of the assumption related to one specific model input. As such, the overall response rate was not a significant limitation of the study” confirming that, out of necessity, much of the information was from an ‘expert panel’ because there was no suitable data in the literature.

An attempt was made to quantify the 'expert panel' levels of uncertainty involved, using the 'Trial Roulette approach' [66]. The clinical experts were provided with a questionnaire to complete in order to quantify their beliefs in a series of parameters. Diagrams provided on the questionnaire represent betting streets, similar to those used on a gaming table. Each column represents a range of potential values for a particular parameter. The clinical experts were instructed that they had 20 gaming tokens to place in some or all of the columns to represent their current belief and uncertainty in the parameters being discussed. Following a brief discussion of the question, the clinician's were asked to start by placing two of the counters at the upper and lower limits of their belief about the parameter value. They were then requested to place the remaining 18 counters so as to express their remaining uncertainty about the particular parameter value. The mean responses are then used to quantify the level of uncertainty involved in the expert's opinion on the question and provide numerical values for the probability distributions assigned to that parameter in the model. This approach was used for unknown parameters such as the malignant transformation rate of potentially malignant disorders and the progression of cancer with time assessed as increasing clinical stage [64]. A multitude of other assumptions and estimates were required to create the complex models developed for this study, each of which is transparently presented in the report but their implications were too often incalculable.

1-1.28 The Cost of Oral Cancer to the NHS

Despite the apparent limitations, the HTA Cost-Effectiveness of Screening for Oral Cancer in Primary Care report [64] provides some data on the approximate total costs over a 3-year period for the management of the stages of OSCC with cost of: precancer £1869; stage I £4914; stage II £8535; stage III £11,883 and stage IV £13,513. This study models total cost to the NHS but does not take into account any patient-related expenses or impact on productivity. The indication being that early detection of OSCC is advantageous in purely monetary terms due to the cheaper treatment required for smaller lesions.

1-1.29 Outcomes of Screening Strategies Modelled in the UK

With the restrictions of the modelling system the HTA Cost-Effectiveness of Screening for Oral Cancer in Primary Care report [64] revealed their “Strategy A” (no screening) was always the cheapest option. The modelled screening strategies were:

(A) No screening - intended to reflect current practice, where lesions may be identified in routine care either via self-referral or through case finding during routine check-ups.

(B) Invitational screen (general medical practice) – all patients registered with a GP are invited for a visual screen. Patients who comply with the invitation receive a visual examination by the GP and any suspicious lesions are referred to secondary care.

(C) Invitational screen (general dental practice) – all patients registered with a GDP are invited for a visual screen. Patients who comply with the invitation receive a visual examination by the GDP and any suspicious lesions are referred to secondary care.

(D) Opportunistic screen (general medical practice) – all patients who attend their GP during the first year receive a visual examination by the GP and any suspicious lesions are referred to secondary care.

(E) Opportunistic screen (general dental practice) – all patients who attend their GDP during the first year receive a visual examination by the GDP and any suspicious lesions are referred to secondary care.

(F) Opportunistic 'high-risk' screen (general medical practice) – all patients who attend their GP and are identified as being at high risk during the first year receive a visual examination by the GP and any suspicious lesions are referred to secondary care.

(G) Opportunistic 'high-risk' screen (general dental practice) – all patients who attend their GDP and are identified as being at high-risk during the first year receive a visual examination by the GDP and any suspicious lesions are referred to secondary care.

(H) Invitational screen (secondary care specialist) – the entire population is invited for a visual screen. People who comply with the invitation receive a visual examination by a secondary care specialist and any suspicious lesions receive a biopsy.

With the principal objective to maximise health gains from available NHS resources the proposal that a cost per QALY value of around £20,000–30,000 is considered value for money by the NHS [67], was adopted. With this restriction, screening strategies B, C, E and H were excluded under all of the modelled outcomes.

The authors conclude opportunistic ‘high-risk’ screening in general dental practice (Strategy G), was the most cost-effective screening model. Screening by GPs (Strategy D and F) was only marginally more expensive despite their lack of specific training and consequently lower assumed sensitivities and specificities in oral visual examination. This was attributed to a higher population coverage in medical practices than dental surgeries [64].

Importantly, opportunistic ‘high-risk’ screening in general dental practice (Strategy G) was only cost effective against no screening (Strategy A) with the unproven assumption that intervention following screening reduced malignant transformation rates by 10-20%, which reportedly cost £15,790 to £18,919 per QALY gained, compared to no screening. If no effect on malignant transformation rates was assumed then the cost was over £20,000 (£22,850) per additional QALY compared with no screening [64].

The model does not allow for any incalculable potential negative effects of screening such as negative impacts on QoL from cancer detection anxiety and treatment related pain and anxiety. Similarly the model does not allow for possible positive psychosocial effects of negative screening for oral cancer.

1-1.30 Oral Cancer Screening Trials in the Literature

The ideal approach to the evaluation of any clinical intervention is the randomised controlled trial (RCT) [68]. However, where there are large gaps in knowledge or where a disease is of low prevalence, making a clinical trial overly costly or of uncertain value, other approaches such as observational or modelling studies, may be of more immediate worth [69]. A PubMed search using the Medical Subject Headings indexing system (MeSH) with Boolean operator combination terms ("Mouth Neoplasms"[Mesh]) AND ("Mass Screening"[Mesh] OR "Early Detection of Cancer"[Mesh]) produces 488 citations from 1963 to 2012. 87 of these are review articles, including an updated Cochrane review [70] and the latest update from a panel convened by the American Dental Association's Council on Scientific Affairs [71] as well as a review by the authors [62] of the HTA Cost-Effectiveness of Screening for Oral Cancer in Primary Care [64].

In 2010 The Cochrane collaboration updated their 2003 review on Screening Programmes for the Early Detection and Prevention of Oral Cancer [70]. This update assesses an additional 330 articles (1719 in total) but due to the stringent criteria for inclusion (http://www.cochrane-handbook.org) still focuses on the single Trivandrum Oral Cancer Screening Study, in Kerala, India [5] as the only RCT to meet these criteria, notwithstanding their own conclusion that this study has a "high risk of bias". Therefore, this Cochrane review concludes "that overall there is not enough evidence to decide whether screening by visual inspection reduces the death rate for oral cancer." Unfortunately, the 'not enough evidence to decide' outcome is often the conclusion from Cochrane reviews due to the scarcity of high quality RCTs in clinical dentistry.

The American Dental Association utilised a different approach to provide clinical guidance, by convening a panel of experts to review the literature and make recommendations. Their latest 2012 Evidence-Based Clinical Recommendations Regarding Screening for Oral Squamous Cell Carcinomas [71] assessed 332 systematic reviews and 1499 other clinical studies, settling on 5 systematic reviews and 4 clinical studies to use as a basis for developing recommendations, which included the Trivandrum Oral Cancer Screening Study [5]. Their conclusion was similar to the Cochrane group with "insufficient evidence to determine if screening alters disease-specific mortality in asymptomatic people seeking dental care."[71]

The authors of the NHS HTA Cost-Effectiveness of Screening for Oral Cancer in Primary Care published their own review on the effectiveness of screening for oral cancer [62] in which 1114 relevant citations were identified and 28 included in the systematic review. Again this included the Trivandrum Oral Cancer Screening Study [5] and again it was concluded that "there are insufficient available data to make an unequivocal determination as to the effectiveness of oral cancer screening programmes".

1-1.31 Limitations of Oral Cancer Screening Reviews

Due to the lack of clinical trial data the highest quality of evidence related to Oral Cancer screening studies appears to come from systematic reviews or meta-analysis of systematic reviews. All of the review articles comment on the heterogeneity of the reported studies including aspects as fundamental as

objectives, study design, geographic location, clinical setting, numbers and characteristics of participants, screening personnel, methods of recruitment and nature of data collected and presented. Even the term ‘oral cancer’ as stated previously can include cancers at sites as different as the lip, salivary glands, tongue and nasopharynx with each having its own unique epidemiology, aetiology, pathology and natural history. It has been suggested that combining data for salivary gland malignancies and OSCC is as rational as grouping breast and lung cancers together simply because of their anatomic proximity [72].

1-1.32 The Trivandrum Oral Cancer Screening Study[1-6]

Widely referenced in the literature and undoubtedly one of the largest and longest running oral cancer screening trials was undertaken in Kerala, in the South-West of India. Trivandrum (Thiruvananthapuram) is the capital of Kerala state and home of the University of Kerala.

The trial employed a cluster randomised controlled study design with an intention to treat (ITT) analysis of 13 population clusters, of which 7 were randomly allocated to 3 rounds of screening (intervention arms), while standard care was provided in the other 6 clusters (control arms).

Trained health workers undertook visual inspection at 3-year intervals in the 7 intervention arms commencing in October 1995 with the first round of screening completed in May 1998, the second completed in June 2002 and the third completed in October 2004. Health workers were trained and provided with

manuals on oral visual examination with colour photographs and descriptions of benign and malignant oral lesions.

Oral visual inspections were performed in daylight with flashlights and intraoral mucosa was visually examined and palpated along with the neck for enlarged lymph nodes. The clinical findings were recorded as normal, non-referable lesions and referable lesions. Interestingly the ability of these trained healthcare workers at detecting oral cancer appears to be equivalent to qualified dentists when compared to studies undertaken in the Western world [64].

Inclusion criteria were healthy participants aged 35 years and older, excluding all those who were bedridden, suffering from tuberculosis or other debilitating diseases and who had been diagnosed with oral cancer prior to entry into the study. In each cluster the number of eligible participants varied from 8000 to 18,500.

Screen-positive individuals were referred for clinical examination by dentists or physicians for confirmation. Oral biopsies were performed on those with clinically confirmed homogeneous leukoplakia, non-homogeneous leukoplakia, OSF and oral cancer. Surgical excision was carried out for leukoplakia wherever possible. All PMDs were regularly reviewed to assess for disease progression. Only 26% of referred lesions actually underwent a biopsy to give a definitive diagnosis. Unfortunately, the reporting does not state whether the screen positive individuals were diagnosed by general medical and dental practitioners who had received formal training or were standardised in the identification of positive lesions.

The control group clusters were also visited by health workers who recorded the same sociodemographic information and measured height, weight, blood pressure and respiratory peak flow measurements. The control group health workers were not trained to undertake an oral examination.

1-1.33 Outcomes of the Trivandrum Screening Trial[1-6]

Of the 96517 eligible participants in the intervention group, 8688 individuals never received the invitation and 174 refused screening. In total, 87655 (91%) were screened at least once, 53312 (55%) twice, and 29102 (30%) three times. As the study employed an ITT analysis bias was minimised despite the significant drop-out rate.

5145 of those screened (5.9%) had referable lesion and 3218 (63%) of these screen positive subjects complied with referral but no indication was given of why so many failed to comply. The control group consisted of 95,356 people of which 80,086 (84%) were assessed.

205 oral cancers and 77 oral cancer deaths were recorded in the intervention group compared with 158 cases and 87 deaths in the control group giving a standardised mortality rate ratio of 0.79 (95% CI 0.51-1.22). The author's extrapolate this finding to suggest that oral cancer screening has the potential to prevent 37,000 oral cancer related deaths worldwide. [5]

The proportion of oral cancers detected at an early stage (i.e. stage I or II) was higher in the intervention arm than the control arm (42% versus 24%). The incremental cost per life-year saved was US$ 835 for all individuals eligible for screening and US$ 156 for high-risk individuals (tobacco or alcohol users). In this study, oral cancer screening by visual inspection was performed for under US$ 6 per person. Taken together the authors suggested that targeted 'high risk' screening of the Trivandrum population was the most cost-effective approach. [6]

Data from 282 oral cancer cases and 1410 matched controls were analyzed using multivariate logistic regression models. Tobacco chewing was the strongest risk factor associated with oral cancer. The adjusted odds ratios for chewers were 3.1 (95% CI = 2.1-4.6) for men and 11.0 (95% CI = 5.8-20.7) for women. Effects of chewing paan with or without tobacco on oral cancer risk were elevated for both sexes. Bidi smoking increased the risk of oral cancer in men (OR 1.9; 95% CI = 1.1-3.2). Dose-response relations were observed for the frequency and duration of chewing and alcohol drinking, as well as in duration of bidi smoking. [1]

Sixty-three percent of 5145 screen-positive individuals complied with referral. Controlling for all other factors, individuals older than 44, and those with more advanced lesions were more likely to comply with referral ($p<0.001$). Individuals living in better housing were less likely to comply with referral (OR 0.79; 95% CI = 0.65-0.95).[2] The significance of the poor referral compliance rate is immense as this indicates many individuals with suspected or frank malignancy remain untreated with potentially no healthcare provision. Furthermore, accurate assessment of the efficacy of the screening programme is impossible as sensitivity

and specificity calculations can only be estimated. Additionally, screening trials in the UK and Western world usually report referral compliance rates approaching 100% [62] indicating significant differences in the characteristics of the population or healthcare system which may invalidate extrapolation of findings from these studies to UK populations.

1-1.34 Cuba's National Oral Cancer Screening Programme

Cuba is the only country in the world to report a national oral cancer screening programme [73]. Oral cancer accounts for 4% of all cancers annually so a national screening programme was commenced in 1984. This covers 12-26% of the population annually and identified 16% of the 4412 new oral cancers diagnosed in Cuba from 1984 to 1990. Additionally tumour staging on diagnosis was improved such that the proportion of early tumours on diagnosis in 1983 was 24% which increased to 49% in 1990. However staging data was available for only approximately half the tumours indentified and there was no change in oral cancer mortality attributable to the screening programme. [73]

1-1.35 Diagnostic Aids for Oral Cancer Detection

With the majority of OSCC being detected at an advanced stage, an inevitable suggestion is that at least some early lesions are missed or ignored by patients and oral health care practitioners. A variety of diagnostic aids to improve the clinician's ability to detect potentially malignant lesions or early tumours have been developed. Some are true screening aids, designed to be used on apparently healthy individuals to help detect early malignancy whilst others aid in case-finding by helping clinicians to diagnose which detected lesions are malignant.

1-1.36 Biopsy and Histopathological Examination

Tissue sampling by scalpel or punch biopsy and subsequent histological examination is the gold standard for diagnosing oral lesions despite the degree of invasiveness resulting in both psychological implications for the patient as well as technical difficulties for the clinician e.g. for large lesions determining the most representative areas to avoid diagnostic errors [74]. Biopsy specimens can also be affected by a number of artefacts resulting from crushing, stretching or incorrect fixation which make histological characterisation substantially more difficult [75]. The subjective inaccuracy of oral epithelial dysplasia assessment in histopathology samples is also well documented [76] and as such, Scully et. al. (2008) recommend that if the pathology report denies malignancy, and yet clinically this is suspected, then discussion with the pathologist and a re-biopsy are invariably indicated [77]. Also, there is significant concern with the potential for the initial biopsy to seed OSCC because of the increased frequency of neck metastasis from stage I and stage II cancers noted after biopsy and the presence of tumour cells in the peripheral blood detected shortly after incisional biopsy [78]. Holmstrup et. al. (2006) [79] even suggest that OSCCs maybe induced by the scalpel incision when PMDs are biopsied. Clearly less invasive and more accurate diagnostic aids are required for the detection of OSCC and PMDs.

1-1.37 Oral Cytology using Brush Biopsy

Exfoliate cytology using brushes (Figure 1-16) has been evaluated since the 1980s as a minimally invasive alternative to scalpel biopsy of oral mucosal lesions. OralCDx (CDx Laboratories, Suffren, NY, USA) is a combination system of transepithelial oral brush biopsy with computer assisted analysis of the sample.

Outcomes are either ‘normal’ or abnormal (‘atypical’ or ‘positive’) with all abnormal results requiring further investigation such as conventional scalpel biopsy and histological assessment to provide a definitive diagnosis.

Figure 1-16: Oral brush biopsy technique for the tongue and buccal mucosa. (From http://www.cdxdiagnostics.com/OralCDx.html)

OralCDx was designed for the examination of visually detected clinical lesions that would otherwise not be subjected to biopsy because the level of suspicion for OSCC was low. The data on accuracy of OralCDx is limited but some very encouraging observations have been reported, such as Sciubba et. al. (1999) [80] showing that 4.5% of 647 of these ‘clinically benign’ lesions when assessed by OralCDx were subsequently diagnosed as dysplastic or OSCC. Sensitivity and specificity calculations for malignancy detection with OralCDx in these ‘clinically benign’ lesions is impossible because, in the published literature, the remainder of these lesions are never definitively assessed by histology, to exclude further concealed dysplasia or malignancy. With current levels of knowledge this is an insurmountable hurdle for ethical reasons as these lesions are believed to be ‘clinically benign’ in nature. Much of the data on the value of OralCDx is therefore derived from its application on clinically suspected OSCC which need conventional biopsy and histology whether OralCDx is applied or not [13].

Another significant issue arises from the inability of OralCDx to determine a diagnosis for detected cellular abnormalities without scalpel biopsy and histology. This would result in mucosal conditions which present with cytologic and morphologic epithelial changes being detected out-of-context as abnormal. For this reason OralCDx cannot reliably be used on common but benign, or at least currently non-malignant, mucosal conditions such as reticular LP [13]. OralCDx application to OSF has not been reported but with the observed histological epithelial changes in OSF a similarly uninformative abnormal result maybe expected.

Despite the immense potential in other clinical scenarios the conclusion must be that OralCDx would neither be a substitute for direct visual assessment of the oral mucosa in a screening trial nor eliminate the need for definitive diagnosis by scalpel biopsy and histological examination.

1-1.38 Experimental Screening Aids

A number of more-or-less experimental techniques for oral cancer detection have been described. These elicit vociferous debate in the literature as to their value as adjuncts to OSCC detection or the subsequent issue of lesion diagnosis, which may be a function of the evident commercial interest as much as specific clinical implication [13, 81-83].

- Vital staining with toluidine blue (e.g. Oratest®)
- Light based techniques (e.g. ViziLite®, VELscope®)

Vital staining and light-based techniques have been evaluated specifically as true screening aids for clinically normal oral mucosa as distinct oral cytology which is an adjunct for aiding diagnosis of visible lesions that appear clinically benign. Overall the light based systems along with toluidine blue, when evaluated as screening or case-finding aids for OSCC, result in the general conclusion that there is insufficient data to support or refute their potential value [13, 60, 71, 77]. Fortunately, a number of potential diagnostic aids are in development, such as DNA ploidy analysis [84], epigenetic biomarkers [85], optical coherence tomography [86] and spectral cytopathology [87]. These are unlikely to become viable diagnostic aids in OSCC screening in the immediate future as the clinical diagnosis ideally needs to be achieved rapidly without resorting to experimental laboratory procedures as the resultant infrastructural and expertise costs would likely render any screening programme non-viable.

In 2012 there are currently no oral cancer screening trials listed on the UK NSC screening portal (http://www.screening.nhs.uk) with the latest review having been completed in June 2010 [60] and not due for review until 2013/14.

1-2.01 AIMS AND OBJECTIVES

OSCC remains an enigma because of the unexplainable disparity between its significant healthcare burden and the potential theoretical ease in decreasing morbidity and mortality with early detection. Most OSCCs develop as visible lesions in the mouth and these detectable early lesions can be easily treated with minimal morbidity. Frustratingly, the majority of OSCCs are still diagnosed at a much later stage when treatment is costly and both morbidity and mortality significant. OSCC therefore remains a promising target for a screening programme despite the evidence for screening efficacy currently being limited.

The data presented suggests that targeted screening of high-risk sub-populations within in the UK is likely to be the most effective methodology. One such group is the South Asian population who are at significantly higher risk for OSCC than the UK population as a whole. They also present with unique PMDs, especially OSF, due to their specific habitual OSCC risk-factor, areca nut extract usage. Therefore, a screening programme aimed at this population would need to account for these cultural variations and utilise the literature on screening trials in South Asia against which the UK screening protocol can be evaluated. Targeted screening activity for OSCC in high risk South Asian populations, conducted in the UK, has not previously been reported in the literature.

The Aims of this study are to:

- Develop a novel targeted screening protocol for OSCC utilising the specific risk factors and disease processes prevalent in a UK based high-risk South Asian population.

- Evaluate outcomes of targeted screening activity for UK based South Asian populations assessed against screening outcomes in their native population compared to trials in the UK population as a whole.
- Evaluate the potential for targeting screening to risk-factor specific PMDs, such as OSF, in the UK based South Asian populations who are at high-risk for OSCC.
- Development of point-of-care immunohistological techniques to eradicate non-compliance with referral for positive screened individuals allowing targeted high-risk OSCC screening activity to be accurately evaluated and ethically implemented.

CHAPTER TWO

DEVELOPMENT OF AN ORAL CANCER SCREENING PROTOCOL TARGETING HIGH-RISK INDIVIDUALS IN TOWER HAMLETS

Chapter 2

Development of an Oral Cancer Screening Protocol Targeting High-Risk Individuals in Tower Hamlets

2-1.01 INTRODUCTION

Oral cancer screening has been suggested as a viable proposition in high risk populations in the UK from extensive modelling exercises [64]. However, these models utilise the aetiological risk factors and available epidemiological data for oral cancer prevalence in the UK population as a whole. South Asian populations in the UK, such as that in Tower Hamlets, are known to be significantly different in terms of socio-economic and cultural influences from the UK population as a whole. For example, the intake of excessive alcohol, a primary contributory risk factor for OSCC in the UK, is uncommon in the South Asian populations of the UK [88]. South Asian populations in their native sub-continent are known to be at significantly higher risk of OSCC because of areca nut extract usage often in conjunction with tobacco [52] and areca compound usage is almost non-existent outside of these populations in the UK. Therefore, the applicability of modelling exercises for the UK population as a whole, to the South Asian sub-population is debatable. A review of the literature reveals no reported OSCC screening trials, specifically targeting this sub-population, in the UK. There are however studies from South Asia including one RCT considered by the Cochrane Oral Health Group, as sufficiently robust to warrant review [70].

2-1.02 Relevance of South Asian Studies to UK Populations

Despite UK based South Asian populations having the same lifestyle related risk factors as their counterparts on the Asian subcontinent, OSCCs multifactorial aetiology suggests that differences in environment and healthcare systems may complicate comparisons. Inequalities in health, related to ethnicity, have been extensively studied because of their potential contribution to the understanding of the role of genetic and acquired risk factors (such as lifestyle and environment) in the development of disease, as well as evaluating the impact of the healthcare system. Additionally, the relative availability of national and international cancer databases has resulted in many of these studies focussing on health inequalities related to cancer [89].

Migrant populations from South Asia resident in the UK are known to have a significantly higher risk of developing OSCC than non-South Asians in the UK [53] and OSCC prevalence is much higher in South Asian countries than the UK [52]. However, the UK based South Asians still display much lower rates of OSCC than their Asian subcontinent peers [53]. This intermediate cancer risk between 'current Western country' and 'native Asian country' has also been shown in the USA [90], and Australia [91]. The finding that South Asians in the UK have a significantly lower risk of all cancers as a whole but specifically experience higher levels of oral cancer, than the general UK population [11] indicates oral cancer specific risk factors are implicated. Moles et. al. (2008) [53] concluded that the lower socio-economic status of many South Asian immigrant populations, in the UK, can only partly account for the differences and suggested that the higher risk of oral cancer reflects the higher consumption of tobacco products and areca nut extracts. They

however acknowledge the limitations of their reliance on the Thames Cancer Registry including potential misclassification in assigning ethnicity on the basis of name and the inability to distinguish sub-ethnic groups within the South Asian population, despite evidence that risk behaviours vary greatly by religion and region of origin [53].

It would appear that in South Asian populations at high risk for OSCC the same aetiological factors are implicated whether living in the UK or South Asia. A targeted screening programme based on the specific lifestyle risk factors, tobacco and areca nut usage, should logically be comparable between UK and South Asian populations, although the prevalence of OSCC is significantly lower in the UK.

2-1.03 The South Asian Population in Tower Hamlets

Tower Hamlets is an Inner London borough (Figure 2-01) and despite its central location in the capital city of England it is ranked as the third most deprived borough in England [54].

Figure 2-01: The London boroughs showing the Inner London position of Tower Hamlets. (Adapted from www.guardian.co.uk)

Approximately 56% of the Tower Hamlets population are reported as ethnic groupings other than white British and one-third of the borough's population is Bangladeshi (Figure 2-02). Totalling nearly 66000 this is the largest Bangladeshi community outside of Bangladesh [54]. In comparison, out of the total population of England and Wales just 0.3% (159,500) is Bangladeshi [92].

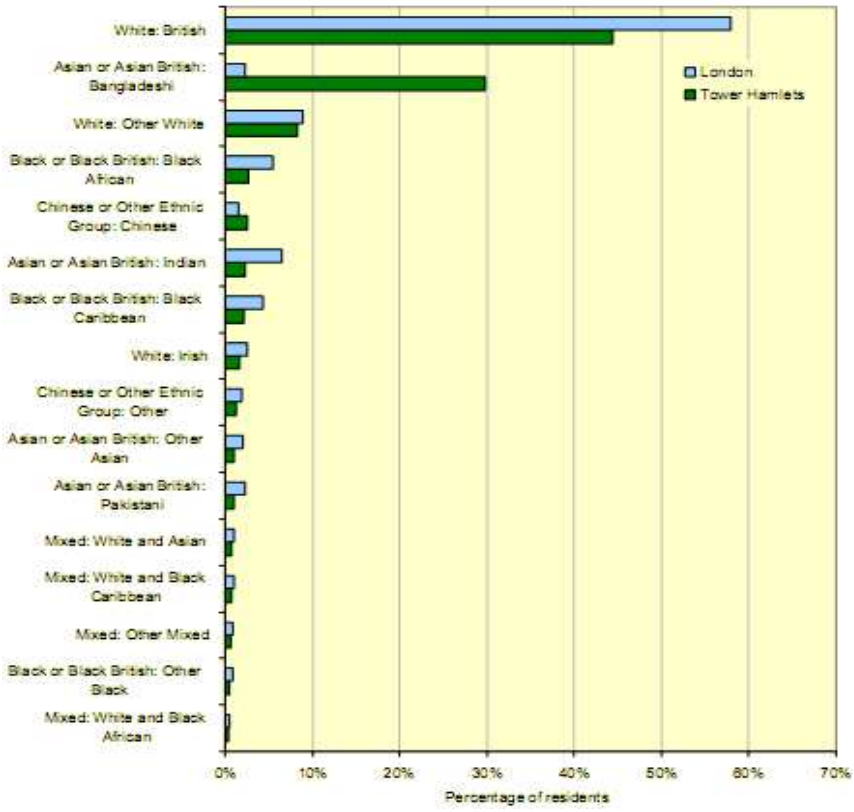


Figure 2-02: 30% of the Tower Hamlets population is Bangladeshi. Adapted from www.towerhamlets.gov.uk and based on Office for National Statistics (2006) population estimates.

Figure 2-03 shows the concentrated nature of the Bangladeshi population predominantly in Tower Hamlets although there is a smaller population in the adjacent borough of Newham to the east, which is ranked as the 6th most deprived borough of the UK [54].

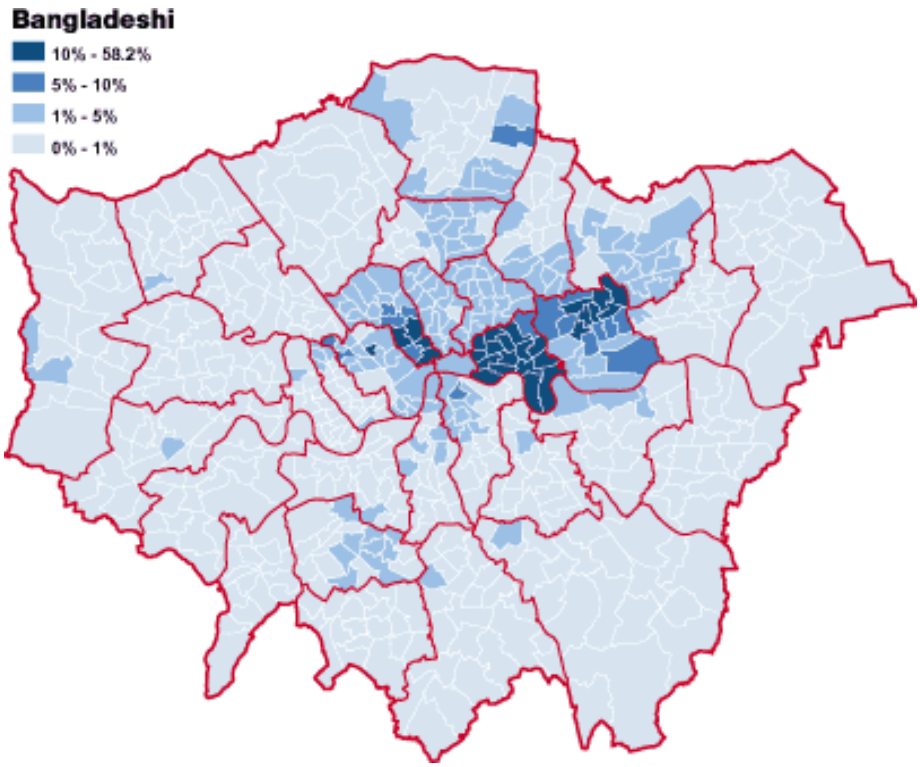


Figure 2-03: Shows the concentrated nature of the Bangladeshi population within London with high proportion of Bangladeshi individuals, predominantly in the borough of Tower Hamlets. Adapted from www.guardian.co.uk based on 1991 Census data.

This is markedly different to the distribution of other South Asian populations such as the Indian and the Pakistani groups in London (Figure 2-04) which are more dispersed and comparatively sparse in Tower Hamlets although there are significant populations in Newham.

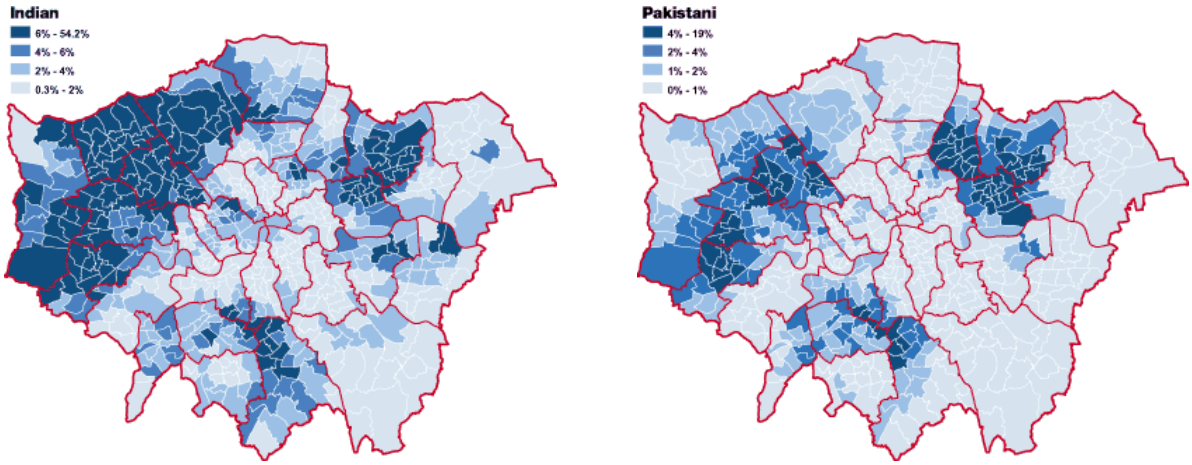


Figure 2-04: The Indian and Pakistani populations in London. (Adapted from www.guardian.co.uk and based on 1991 Census data.)

Within the borough of Tower Hamlets the Bangladeshi community is further concentrated to specific wards. These wards are shown in Figure 2-05, whilst Figure 2-06 shows the distribution of the Bangladeshi population to be most highly concentrated within the Whitechapel, Spitalfields and Banglatown, Bethnal Green South wards to the east of the borough and the Mile End East, Stepney Green and Limehouse wards in the centre of Tower Hamlets as well as the Bromley by Bow ward to the east.

Figure 2-05: The Inner London borough of Tower Hamlets comprises a number of geographically distinct wards. (Adapted from www.towerhamlets.gov.uk)

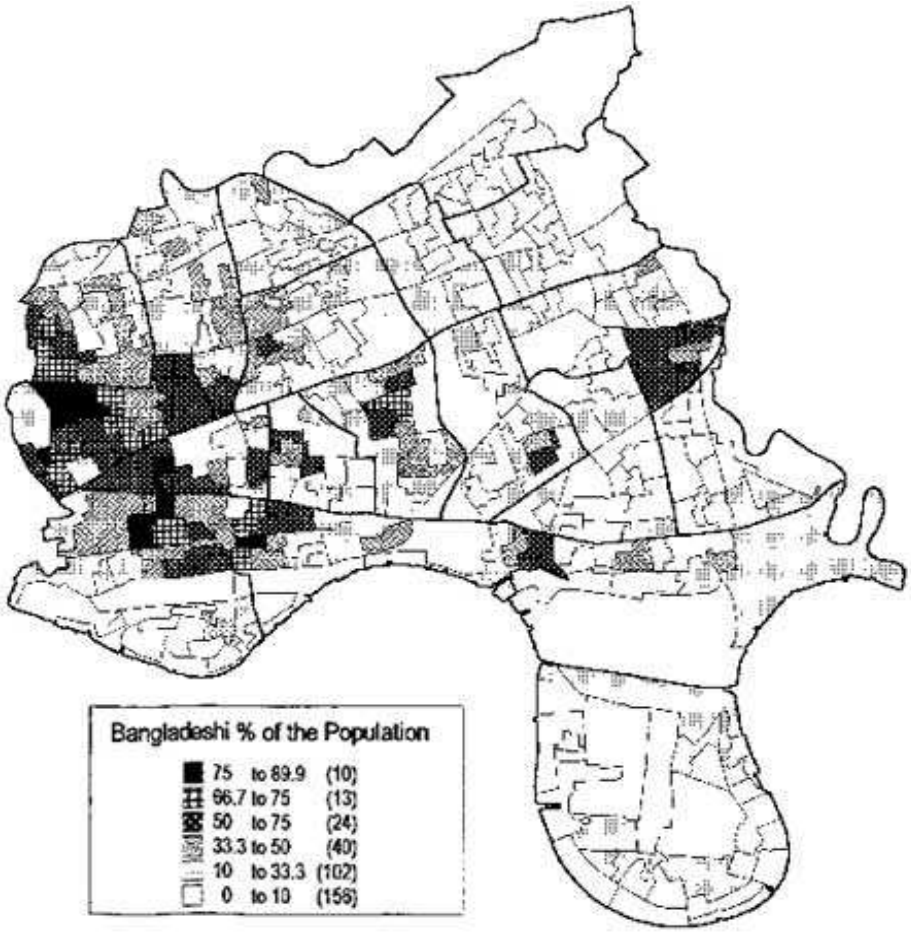


Figure 2-06: Distribution of people of Bangladeshi origin in Tower Hamlets based on 1991 census data. (Adapted from www.nationalarchives.gov.uk)

Tower Hamlets is an ideal setting for conducting a screening project because it offers the opportunity to specifically target a large and relatively homogenous Bangladeshi South Asian community residing in a relatively small geographical area.

2-1.04 Risk Factors for Oral Cancer

In 2004 the annual Health Survey for England (HSE) focused on the health of minority ethnic groups [93]. The sample was designed to yield additional interviews with members of minority ethnic groups, the largest of which is South Asian and comprises Indian, Pakistani and Bangladeshi [54]. The Bangladeshi community, in particular, exhibits a high prevalence of a number of oral cancer risk factors, including smoking, chewing tobacco and chewing areca nut but not alcohol consumption when compared with the general adult population [88, 93]. 40% of Bangladeshi men report being current smokers, compared to 24% of the general male population whilst Bangladeshi women are substantially less likely to report being current smokers compared to women from the general population (2% compared to 23%). Estimates of chewing tobacco or areca nut use range from 16% of Bangladeshi women using paan with tobacco and 13% paan without tobacco [93] up to 48.5% of a sample of Bangladeshi women resident in Tower Hamlets using paan with tobacco [88].

As expected the Bangladeshi community of Tower Hamlets shows significantly different OSCC risk factors from the UK population as a whole but comparable to other South Asian populations. Therefore, the outcomes of a screening programme in this population maybe better compared against reports of screening

undertaken on the Asian subcontinent than other UK trials particularly as screening programmes conducted in Bangladesh itself are not available in the literature.

With a South Asian population of approximately 75,000 in Tower Hamlets [54] and a reported prevalence of OSCC approaching 20 per 100,000 in the South Asian population group [52] it would be likely that 15 OSCCs were present in the study population. A case-finding protocol targeting those individuals at highest risk would be expected to effectively detect these lesions with the need to only examine a small proportion of the total population. However there are many known factors which may interfere with the effectiveness of the screening programme.

2-1.05 Uptake of Screening Services

The models for screening evaluated by Speight et. al. (2006) [64] suggested that oral cancer screening by primary care general dental practitioners maybe the most cost-effective protocol. As with risk factors for OSCC the Tower Hamlets Bangladeshi population appears to be significantly different to the UK population as a whole. In a study of 158 Bangladeshi medical service users in Tower Hamlets, Pearson et. al. (2001) [94] showed that just 20% were regular dental attendees with another 13% having utilised an Emergency Dental Service in the past year and 25% having never visited a dentist [94]. A variety of barriers to access have been postulated including language difficulties, a symptom orientated view of service and domestic isolation of women [95].

In the UK's two national screening programmes, breast and cervical cancer, screening services are consistently poorly utilised by the UK South Asian population [96]. Amongst all ethnic minority groups Bangladeshi's were the least likely to have had any type of cancer screening with only 28% of eligible Bangladeshi women reported to have had a cervical smear [23]. The main reasons reported for low uptake were a lack of knowledge about screening services, language barriers, inaccurate screening registers, including poor awareness of minority ethnic naming systems, all compounded for Asian women by extended visits to the Indian subcontinent and a lack of referral/recommendations by healthcare professionals [97]. Studies attempting to improving the uptake rates are limited but personal visits have been shown to be more effective than posted leaflets [98] for cervical but not breast cancer screening [99, 100]. Complex, multi-strategies do consistently appear to improve uptake of cancer screening by ethnic minorities but are never cost-effective to undertake [101, 102]. Additional interventions include practice receptionist training, follow up letters in various languages, offers of transport and health advocates on site.

In conclusion, it appears that an OSCC screening strategy utilising existing primary care dental services is likely to be ineffective within the Tower Hamlets Bangladeshi high-risk population and a multi-strategy approach will be required to ensure adequate uptake.

2-1.06 Protocols for Oral Cancer Screening in South Asia

The Trivandrum Oral Cancer Screening Study [5] utilised a community based approach whereby screening was carried out in the subject's home. Participants

were screened with minimal intrusion into their normal daily routine, as the highest risk population is unlikely to utilise existing care services, and this study achieved a 91% uptake rate. In the UK a number of procedural and legal issues could be envisaged with a similar protocol and other difficulties, such as language and cultural barriers, which may not be issues in the Asian subcontinent trial.

2-1.07 The Practice of Dentistry

There is a regulatory issue of utilising individuals without dental qualifications to undertake oral screening. A direct visual examination of the oral mucosa with the express intent of detecting OSCC would be construed as the 'practice of dentistry'. The General Dental Council of the UK (www.gdc-uk.org) states "The Dentists Act 1984 makes it a criminal offence for a person who is not a registered dentist or a registered dental care professional to practice dentistry, or hold themselves out - whether directly or by implication - as practicing or as being prepared to practice dentistry." Therefore, registered dental practitioners who can take professional responsibility for their screening services, would be required in the UK. This has the secondary advantage of allowing screened subjects to be given personalised advice on their oral health but recruitment of dentists would necessarily incur higher cost than utilising other healthcare practitioners.

2-1.08 Electoral Registers

Use of electoral registers for sampling minority ethnic groups is known to be very expensive in terms of time and labour and in addition the completeness of the electoral register for minority ethnic groups has also been questioned [103]. Pearson et. al. (2001) [94] when attempting to sample the Bangladeshi population reported

issues with Local Research Ethics Committee's expressing concern about approaching subjects at home having gained information from other health resources such as medical practitioners. The implication being that only subjects responding to letters of invitation could be approached in their home. This would likely result in a very small and biased sample as studies with this methodology in Bangladeshi communities often have response rates as low as 17% [104].

2-1.09 Language and Cultural Barriers

Poor linguistic competence is a major barrier to access for the Tower Hamlets Bangladeshi population as only 27% of this population report they can understand English "well" or "fairly well" [105]. Studies in the Bangladeshi population of Tower Hamlets have shown that Sylheti speaking interviewers are vital to improve uptake of services [94]. Other measures that have been reported as useful to improve access include: materials developed and tested for specific cultural, ethnic, and linguistic groups; translation services including those of legally binding documents (e.g. consent forms), hospital signage, health education and public awareness materials and campaigns and ethnic media in languages other than English (e.g. television, radio, internet and newspapers) [97].

Various aspects of culture can influence successful healthcare delivery to ethnic minority populations and this is likely to be a more persistent problem than language in highly transient populations such as the Bangladeshi community of Tower Hamlets. Cultural issues include: how illness, disease, and their causes are perceived; their behaviour in seeking health care and their attitudes toward healthcare providers as well as the views and values of those delivering health

care [97]. Therefore, a screening programme targeting the Bangladeshi population needs to be culturally as well as linguistically competent.

2-1.10 Summary

Conventional models of targeted high-risk screening for oral cancer in the UK are unlikely to be suitable for the Bangladeshi population of Tower Hamlets due to the specific socio-economic and cultural risk factors prevalent within this population. We propose that a protocol specifically addressing these influences is required for an effective OSCC screening programme in this South Asian population. In addition, the assessment of outcomes of screening activity in the Tower Hamlets Bangladeshi population is better assessed against screening programmes undertaken in other South Asian populations than comparison to other UK studies.

2-2.01 DEVELOPMENT OF THE SCREENING PROTOCOL

2-2.02 The Mobile Dental Unit

In order to facilitate screening in the community the Tower Hamlets Primary Care Trust's mobile dental unit was utilised. The mobile dental unit is a specially adapted van which has a fully functioning dental unit with chair and light powered by a diesel generator (Figure 2-07).

Figure 2-07: The Tower Hamlets Primary Care Trust Mobile Dental Unit. External and internal images showing the fully functional, private and secure dental unit.

This vehicle has been used within the Community Dental Service to provide routine dental services within the borough and appears to be a well accepted. It is a recommended means of improving access to dental services within the NHS as shown by Tower Hamlets Primary Care Trust's own evaluation reports [106] although robust evidence for these claims is not available. In its implementation for OSCC screening the mobile dental unit would be the field base from which bilingual link workers would approach high-risk individuals and invite them for screening by the dentist. The dentist would remain on the mobile dental unit to utilise the reclining dental chair, dental light and mouth mirrors for accurate visual examination, aided

by the availability of dry gauze, ‘3 in 1’ air and water and the aspirator to wipe, wash or dry the mucosa as required. The privacy afforded by the mobile dental unit is required when assessing the patient’s complaints or relevant medical history and lifestyle risk factors. Cross infection control during examination could be maintained on the mobile dental unit at levels comparable with conventional dental clinics.

2-2.03 The Community Advisory Group (CAG).

To develop a delivery structure that is targeted but culturally appropriate as well as feasible, a Community Advisory Group was convened which comprised local stakeholders from Tower Hamlets Primary Care Trust (THPCT), Smoking Cessation and Dental Access teams; Barts and The London Cancer Services including Oral Medicine and Oral and Maxillofacial Surgery teams as well as practice nurses and patient and community representatives. This CAG had previously been involved in the ‘Bangaldeshi Stop Tobacco’ project developed in the Tower Hamlets area. The CAGs primary objective in the development of the screening protocol was to offer guidance on the promotion of oral cancer screening activity amongst the Bangladeshi community and the development of accompanying oral cancer awareness literature. Additionally, the CAG was involved in determining the screening locations of the mobile dental unit to maximise the number of high risk individuals assessed in the population. More formal qualitative research was conducted with the target population, by means of focus group discussions and key informant interviews, to further aid development of the oral cancer screening activity and accompanying literature. This was

conducted by members of the Bangladeshi Stop Tobacco project in combination with their tobacco cessation small group and individual help sessions.

2-2.04 Advertising and Promotion

Information campaigns for oral cancer awareness have been evaluated in the literature with some evidence for leaflets leading to increased knowledge although predominantly in those of a higher educational level and younger age [107]. News programs and TV campaigns show little improvement in awareness of oral cancer, at least in American populations [108]. In Tower Hamlets, Sylheti is the regional variant of Bengali spoken by 98% of Bangladeshi people [105] but Sylheti does not have a written form of its own and Bengali is the language of literacy amongst this population and only 16% can read or write it [105]. With the knowledge that multi-strategy approaches consistently improve uptake of cancer screening by ethnic minorities [101, 102] promotion of the screening campaign and oral cancer awareness would involve specifically designed leaflets (Figure 2-08) as well as newspaper promotions and local radio advertising campaigns along with crucially timed announcements at the local mosques and community centres.

Figure 2-08: Cancer Research UK leaflet and poster in English and Bengali (from www.cancerresearchuk.org)

2-2.05 Identification of Screening Sites

The CAG was tasked with identifying sites where a significant number of high risk individuals would be approachable and the mobile dental unit could be sited.

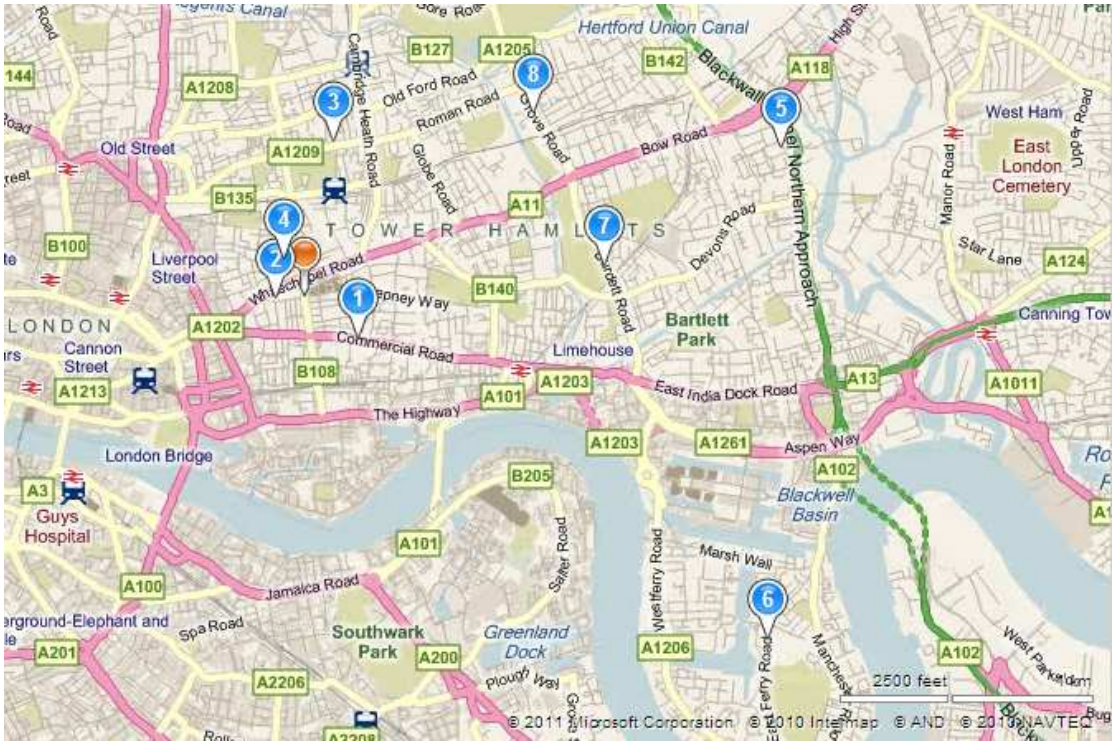


Figure 2-09: The sites in Tower Hamlets chosen for screening activity.
(1) Watney Market, (2) East London Mosque, (3) Bethnal Green Road, (4) Brady Arts Centre, (5) Bromley By Bow Centre, (6) Asda, Isle of Dogs, (7) Mile End Leisure Centre and (8) Mile End Park. (Map adapted from www.bing.com).

Figure 2-09 shows the locations chosen for their proximity to local facilities and travel routes. It was determined that most screening sessions should be in the wards with the highest proportion of Bangladeshi residents (Fig 2-06) therefore screening sites at Watney Market, The East London Mosque, Bethnal Green Road, Brady Arts Centre are in the western wards of Whitechapel, Spitalfields and Banglatown and Bethnal Green South. Mile End Leisure centre was chosen to target the populations of the central wards of Mile End East, Stepney Green and Limehouse whilst the Bromley by Bow location targeted the Bangladeshi population in the eastern ward of Bromley by Bow. It was suggested by the CAG

that although the available Census population data (Figure 2-06) didn't support screening in the southern wards of Millwall and Blackwall and Cubitt Town on the Isle of Dogs these areas may now have significant Bangladeshi populations. Asda, Isle of Dogs was chosen as the location to target these individuals. Finally, a screening session at Mile End Park was required to target the northern wards of Tower Hamlets. Funding for 10 screening sessions was provided by Cancer Research UK based on the likely throughput of screened patients and relative density of high-risk Bangladeshi populations.

2-2.06 Screening Criteria

Oral cancer screening would involve a brief history to elicit any relevant presenting complaints or medical history followed by visual examination of the soft tissue of the oral cavity and oropharynx, and palpation of the neck for lymph node involvement. Screening would be conducted by registered dental practitioners recruited from the Community Dental Service in Tower Hamlets who had experience of dental treatment of the Tower Hamlets population. A refresher training session was provided to ensure compliance with the National Institute of Clinical Excellence guidelines on referral of suspected cancer [10] which determines a positive screen for OSCC to be:

- An unexplained ulcer or lump in the mouth lasting more than three weeks
- Unexplained red or white patches inside the mouth that are painful or swollen or bleeding.
- Persistent undiagnosed symptoms in the mouth lasting longer than six weeks.

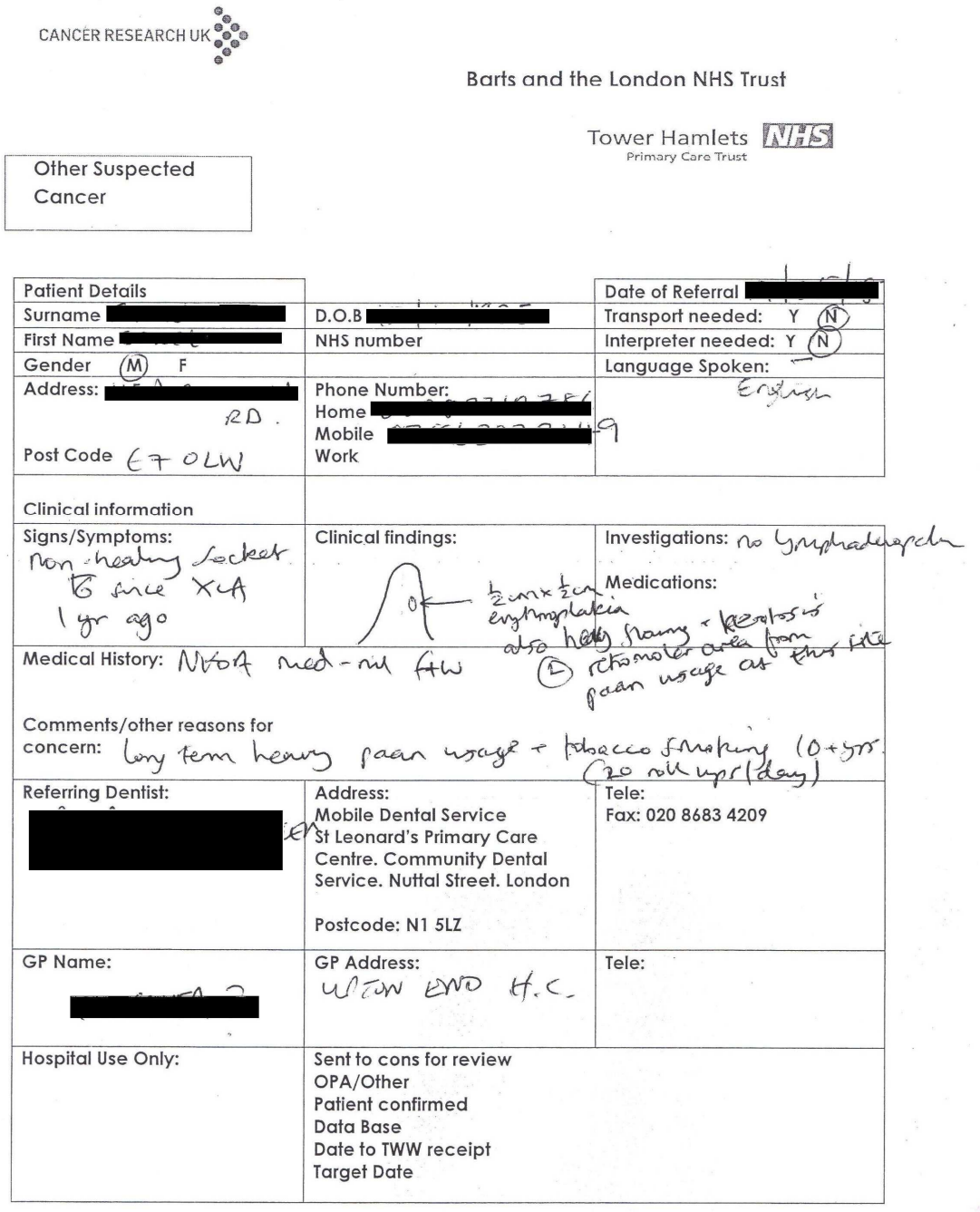
- An unexplained lump in the neck which has recently appeared or a lump which has not been diagnosed before that has changed over a period of three to six weeks.

Minimal data collection on the individuals screened was attempted unless referral was indicated. This was a consensus decision of the CAG as it was felt that people were more likely to attend an anonymous drop-in service and the flow of patients would be quicker thereby removing some potential barriers to access in the Bangladeshi population. Preliminary data suggested that each patient could be screened in an average of 3 minutes but referrals would take significantly longer as the dentist would endeavour to reassure the patient and answer any questions to minimise anxiety during the wait for definitive ff. Data collection was approved by The East London and the City Research Ethics Committee and the Joint Research and Development Office of Bart's and The London (REC 08/H0701/98). Data analysis utilised Excel 2003 (Microsoft, USA) with the StatPro add-on (Kelley School of Business, Indiana University). To allow for minimal data collection and anonymity all participants gave verbal and implied consent to take part in screening but written consent was obtained from those who needed and agreed to be referred.

2-2.07 Development of Referral Pathways

Screen positive individuals were referred directly from the mobile dental unit to the Department of Oral Medicine, Bart's and the London Dental Institute for further investigation (Figure 2-10). The referral followed an urgent two-week referral pathway for suspected cancer and additional capacity was created on clinics

specifically for these referrals. Furthermore, individuals could also be referred directly to local stop tobacco services for cessation support.



CANCER RESEARCH UK

Barts and the London NHS Trust

Tower Hamlets NHS
Primary Care Trust

Other Suspected Cancer

Patient Details		Date of Referral [redacted]
Surname [redacted]	D.O.B [redacted]	Transport needed: Y (N)
First Name [redacted]	NHS number	Interpreter needed: Y (N)
Gender (M) F		Language Spoken: English
Address: [redacted] RD. Post Code E7 0LW	Phone Number: Home [redacted] Mobile [redacted] Work	
Clinical information		
Signs/Symptoms: Non-healing socket R since XLA 1 yr ago	Clinical findings: ½cm x ½cm erythroplakia also heavy staining + keratosis (L) retromolar area from paan usage at this site	Investigations: no lymphadenopathy Medications:
Medical History: NKDA med-nil FH		
Comments/other reasons for concern: long term heavy paan usage + tobacco smoking (0+yrs (20 roll ups/day)		
Referring Dentist: [redacted]	Address: Mobile Dental Service St Leonard's Primary Care Centre. Community Dental Service. Nuttal Street. London Postcode: N1 5LZ	Tele: Fax: 020 8683 4209
GP Name: [redacted]	GP Address: WTON END H.C.	Tele:
Hospital Use Only:	Sent to cons for review OPA/Other Patient confirmed Data Base Date to TWW receipt Target Date	

Figure 2-10: The referral form for suspected oral cancer directly from screening sessions to the Oral Medicine Unit at The Royal London Hospital.

2-2.08 Determination of Inclusion Criteria

Individuals were actively recruited by the bilingual link workers at each screening site based on the proposed risk factors for oral cancer in South Asian populations:

- Age 35 years or over
- South Asian ethnic origin
- Practising one or more of the risk behaviours of smoking or chewing tobacco, chewing areca nut compounds (paan) or alcohol usage.

Individuals falling outside these inclusion criteria were not actively recruited by the link workers but were not excluded from screening if they wished to attend.

2-2.09 The Delivery Process

The targeted oral cancer screening of the Bangladeshi population of Tower Hamlets involved 10 screening sessions during 2006. The project and protocols were then to be evaluated against the literature on trials completed in South Asia and the UK.

Screening was undertaken using a mobile dental unit which provided a clean, safe and confidential environment for examination of the illuminated oral mucosa in the supine position with the availability of all the instruments and amenities that would be present in a permanent dental clinic. Use of the mobile dental unit addressed all of the CAGs issues arising from pre-trial attempts at mucosal examination with portable equipment which included: inconsistent lighting intensity; patient confidentiality and modesty concerns; difficulty gaining adequate oral mucosal access in the upright position with associated concerns about examiners posture if

screening for an entire working day and lastly issues with maintaining appropriate levels of cross-infection control.

Ensuring the cultural acceptability of the oral cancer screening activity was the role of bi-lingual (English/Sylheti) advocates with experience of health promotion activity in this community. In addition to providing language support they were involved in actively recruiting participants to the oral cancer screening sessions and onto tobacco cessation services. Advocates further facilitated the referral process and attended outpatient appointments at the secondary referral centre, if required. This ensured as many patients as possible complied with referral.

2-3.01 RESULTS

In 2006 the oral cancer screening programme, targeting high risk individuals in the Bangladeshi community of Tower Hamlets, was commenced. A total of 10 screening sessions were undertaken and each session was approximately seven hours in duration and undertaken between 0930 and 1630 with flexible break times dependent on the flow of patients.

2-3.02 Numbers Screened

In 10 screening sessions a total of 485 individuals were screened, an average of 48 people per session, although this varied from 19 on the quietest session at Bromley by Bow to 82 on the busiest on Bethnal Green Road (Table 2-01). The Bromley by Bow sessions was also the only day on which the weather was wet and may have contributed to the scarcity of individuals recruited for screening.

Table 2-01: Numbers screened and referred from each screening session.

Screening Session		Location	Screened	Referred	Dentist
2006	Wednesday 14th June	Brady Arts Centre	57	3	AL
	Saturday 24th June	Asda, Isle of Dogs	68	0	AL
	Friday 30th June	East London Mosque	61	9	AR
	Saturday 1st July	Bethnal Green Road	82	2	AL
	Saturday 15th July	Mile End Leisure Centre	45	0	AL
	Saturday 29th July	Mile End Park	42	1	AL
2007	Saturday 30th June	East London Mosque	51	1	AL
	Saturday 16th July	Watney Market	27	4	AR
	Wednesday 20th July	Bromley By Bow Centre	19	0	AL
	Saturday 23rd July	Brady Arts Centre	33	0	DR
		TOTAL 10 SESSIONS	485	20	

2-3.03 Referrals to Secondary Care Services

Of the 485 people screened 20 (4.1%) were referred with suspected cancer for further investigation (Table 2-01).

2-3.04 Referrals by Screening Site

Table 2-01 shows 3 screening sites yielded no referrals although 132 individuals were screened. One session at Brady Arts Centre also yielded no referrals despite 33 screenings although the earlier session at this site by a different dentist screened 57 and referred 3 (Table 2-01). The Asda, Isle of Dogs site resulted in 68 individuals screened but none referred. In contrast at the Watney Market site just 27 were screened but 4 (15%) were referred. East London Mosque was visited on two occasions resulting in 112 screenings and 10 referrals (9%). Therefore, 50% of all referred patients were screened at the East London Mosque site but only 22% of all screened individuals were seen at this location and 20% of referrals were from Watney market despite just 6% of all screenings being at this site (Figure 2-11).

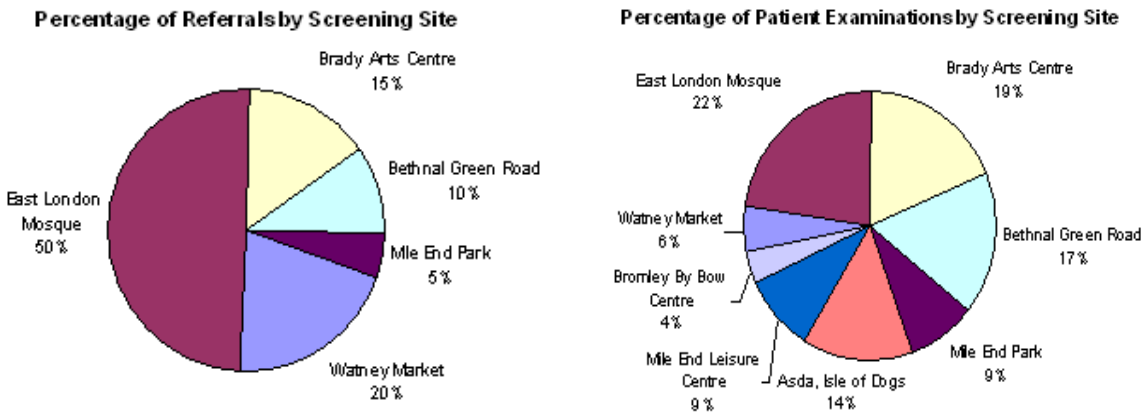


Figure 2-11: Proportion of individuals screened at each site (Total screenings 485) and percentage of referrals by screening site (Total 20 referrals).

2-3.05 Referrals by Dentist

Dentist AR referred 13 (15%) of 88 screened at two sessions and Dentist AL, despite screening at seven sessions referred just seven (2%) of 364 individuals screened. Dentist DR screened 33 in one session and none were referred (Figure 2-12).

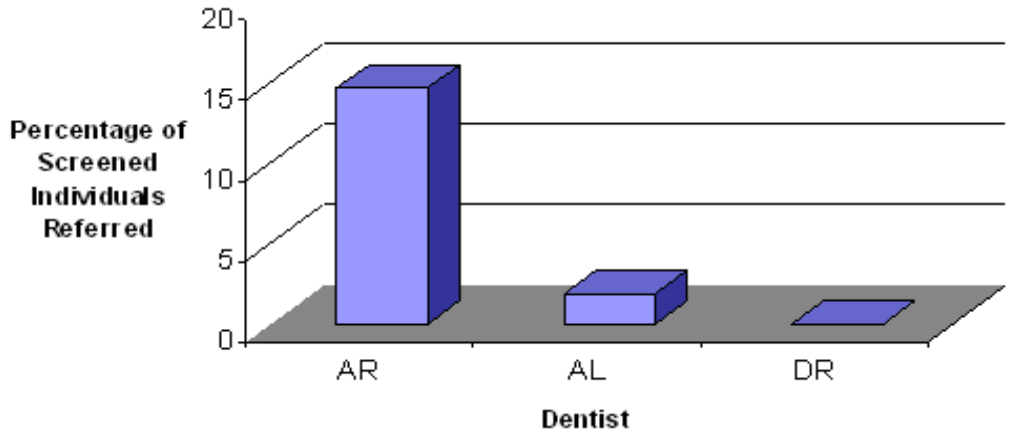


Figure 2-12: Percentage of screened patients referred by Dentist. (Total 20 referrals).

2-3.06 Outcomes of Referrals to Secondary Care Services

Despite the involvement of the advocates in chasing referrals and offering to attend appointments with the positively screened individuals, six patients (30%) of the 20 referred failed to attend the secondary referral centre for definitive diagnosis (Table 2-02). Attempts were made to make contact with these individuals by both telephone and post and then by home visit. For ten of the 14 referred patients were a definitive diagnosis was determined either following biopsy and histological examination (8 patients) or from clinical examination by the supervising Oral Medicine consultant where biopsy was not indicated (2 patients).

*Table 2-02: Definitive diagnosis and outcome after referral to secondary care services of positively screened individuals. (DNA= did not attend secondary referral centre; *****= definitive diagnosis confirmed by histology). Four diagnosed with potentially malignant disorders are highlighted.*

Patient	Primary Diagnosis	Outcome
ALbra-1	**oral submucous fibrosis***	review
ALbra-2	hyperkeratosis*	review
ALbra-3	**mild/moderate dysplasia***	review
ARelm-1	DNA	-
ARelm-2	hyperkeratosis	discharge
ARelm-3	hyperkeratosis	discharge
ARelm-4	DNA	-
ARelm-5	hyperkeratosis	discharge
ARelm-6	hyperkeratosis*	review
ARelm-7	physiological pigmentation	discharge
ARelm-8	hyperkeratosis*	review
ARelm-9	DNA	-
ALbgr-1	**mild/moderate dysplasia***	review
ALbgr-2	DNA	-
ALmep-1	DNA	-
ALelm-1	**severe dysplasia***	review
ARwat-1	hyperkeratosis	discharge
ARwat-2	hyperkeratosis*	discharge
ARwat-3	DNA	-
ARwat-4	hyperkeratosis	discharge

2-3.07 Definitive Diagnosis

Figure 2-13 shows the definitive diagnosis for the 14 screen positive referrals who attended the referral centre. There were no OSCC detected during this screening project however, three referred patients were diagnosed with dysplastic lesions one of which was severe dysplasia and the others mild/moderate dysplasia. In view of the associated risk factors these lesions were surgically excised and the patients remain under review as well as being referred to ancillary services to aid tobacco/paan cessation.

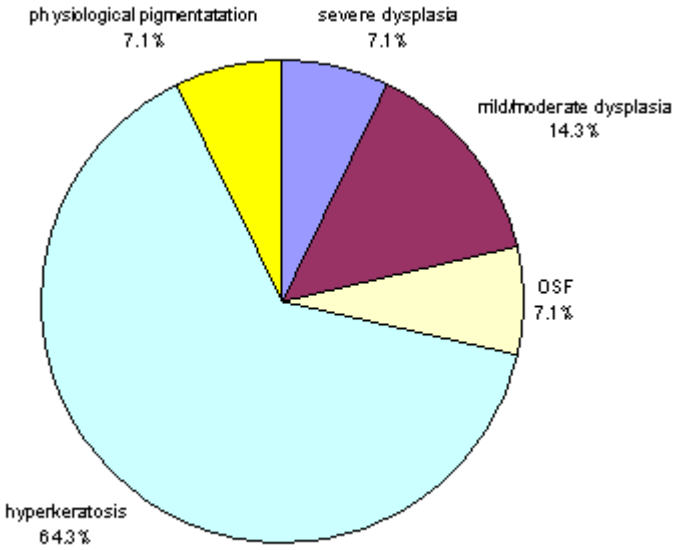


Figure 2-13: Definitive diagnosis for 14 positively screened patients attending the secondary care centre.

One referred patient was diagnosed with the potentially malignant mucosal disorder OSF and remains under review for evidence of malignant change as well as being helped to reduce tobacco and paan usage. In total four out of 14 (28.6%) of the positive screened patients were diagnosed with PMDs (Figure 2-14).

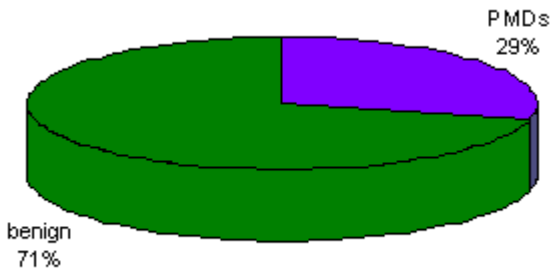


Figure 2.-14: Potentially malignant disorders (PMDs) and benign lesions within the positively screened population who attended for biopsy. (Total 14)

Of the 14 positively screened patients who attended the secondary referral centre, eight (57%) underwent scalpel biopsy and histological examination. The other 6 were diagnosed clinically by the Oral Medicine specialist as benign lesions with no indication for histological evaluation (Figure 2-15).

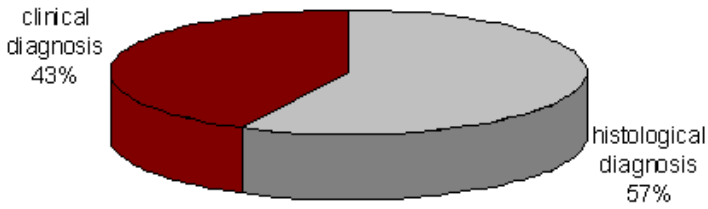


Figure 2-15: Method of achieving the definitive diagnosis for screen positive individuals at the tertiary referral centre. (Total 14)

These latter six were also able to be discharged from further follow-up along with one of the biopsy patients diagnosed with hyperkeratosis. Ten patients were diagnosed with benign lesions, nine of which were hyperkeratosis and the other presenting with physiological pigmentation. However, of these 10 patients with confirmed benign lesions only seven with minimal risk factors and access to appropriate follow-up within primary dental care services, were able to be discharged from further review in the Department of Oral Medicine. Therefore, seven out of 14 (50%) of positively screened individuals who attended for diagnosis remain under review at one year after the last screening session (Figure 2-16).

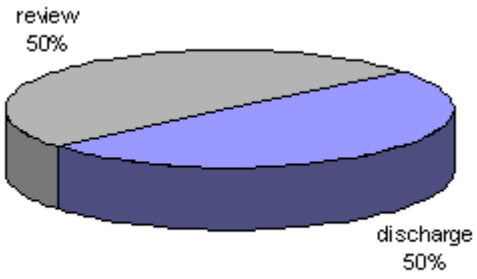


Figure 2-16: Outcome of referral for the positively screened population who attended the secondary referral centre at 1 year after the last screening session. (Total 14).

Two of the four patients diagnosed with PMDs were screened at Brady Arts centre with the others at East London Mosque and Bethnal Green Road respectively.

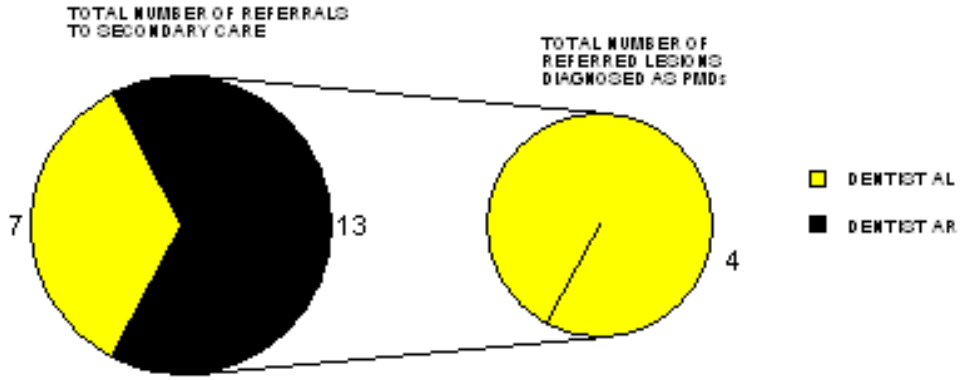


Figure 2-17: The proportion of all referred patients with lesions diagnosed as PMDs by referring dentist.

Dentist AL referred seven individuals in total of which five attended the secondary referral centre and were sent for biopsy. Four of these five (80%) were diagnosed with potentially malignant disorders. Dentist AR referred 13 in total of which nine attended and three underwent a biopsy but all were diagnosed with benign lesions (Table 2-02 and Figure 2-17).

2-4.01 DISCUSSION

Of the 435 people screened in ten sessions, 20 (4.1%) were referred for further investigation of suspicious lesions. This referral rate is broadly consistent with that reported in previous oral cancer screening initiatives [62, 109] and in particular is of the same order of magnitude as the 5.9% referral rate reported in the Trivandrum Oral Cancer Screening Study [5]. This would suggest the screening protocol overall is comparable to other programmes in the published literature.

2-4.02 Gold Standard Outcomes

Despite 435 individual screenings of a high risk population for oral cancer there were no cases of OSCC detected. Therefore, it is impossible to assess the results on the basis of the ideal gold standard for one-off oral cancer screening programmes, which is histologically confirmed OSCC. The fact that 6 (30%) of the 20 individuals referred to the secondary referral centre did not attend for further investigation of their suspicious oral mucosal lesions makes assessment of a gold standard outcome even less accurate. However, there are also insurmountable ethical issues in obtaining the true sensitivity of a screening process by histological gold standard because all screened individuals theoretically need a biopsy for histological diagnosis confirming either OSCC or not. Simply determining a histological diagnosis for all referred lesions only, would be ethically more acceptable but risks introducing verification bias. For this reason most studies determine screening success by some form of ‘soft’ gold standard such as the number of positively screened individuals or the proportion subsequently diagnosed (either clinically or histologically) with PMDs or OSCC [109].

2-4.03 Evaluation of Clinical Outcomes

Utilisation of the soft gold standard outcome of a PMD diagnosis with the 14 patients who attended for definitive diagnosis yields a PPV of 28.6% (4 true positives out of 14). As these individuals present with significant risk factors for OSCC this may be regarded as a more suitable gold standard for screening outcomes than simply being referred to the secondary care centre, thereby eliminating those referred with benign lesions. This position has further advantages in that some severe dysplastic lesions in these high-risk populations are likely to be managed in the same manner as a small primary OSCC without nodal spread or metastases i.e. a $T_1N_0M_0$ malignancy. These lesions are either excised during the biopsy process or surgically or laser ablated if more widespread. The patient would then remain under regular review with efforts made to educate and minimise associated risk factors. Further biopsies are required whenever the mucosal appearance changes to catch any malignant transformation early.

2-4.04 Referral Compliance

Only 14 (70%) of the 20 individuals referred to the secondary referral centre attended for further investigation of their suspicious oral mucosal lesions. This was despite the best efforts of the advocates to phone and visit the positively screened individuals as well as offering to attend at their hospital appointment. Reasons for non-attendance were investigated in patients who initially refused to comply with referral but subsequently did attend. Causes for initial non-attendance included language barriers, non-receipt of appointment letters and difficulty attending the hospital. This indicates that the model of screening, in a community setting with

ethnically-matched advocates was able to overcome some specific barriers to access that still exist in the hospital. However, there is significant room for further improvement in compliance with referrals. Overall, the compliance rate is comparable to the 63% referral compliance reported in the Trivandrum Oral Cancer Screening Study [5] . In contrast, screening programmes in the UK usually report very high levels of compliance, approaching 100% [62]. This suggests that the Tower Hamlets Bangladeshi population is behaving more akin their South Asian peers when responding to oral cancer screening and supports the notion that studies in these populations are more comparable than studies in UK populations.

2-4.05 Potential for Inter-Examiner Variability

Although inter-examiner correlation was not directly assessed in the protocol the differences in referral rate indicates a potential for significant variability. Dentist AL referred 1.9% of screened individuals and 80% of these were subsequently diagnosed with potentially malignant lesions whilst dentist AR referred 14.8% of screened individuals, of which, all who complied with referral were diagnosed with benign lesions. This could be due to the different populations screened on different days and at different sites but qualitative post-screening evaluation indicated this was also due to difficulty in diagnosing mucosal lesions in people who chew areca and tobacco products, where the mucosa is heavily stained. Additionally, there is ambiguity within the NICE guidelines on referral of suspected malignancy and in particular the one-off nature of screening does not allow for lesions to be observed before referral, as is possible in other primary dental care settings. This variability is consistent with the literature on screening sensitivity, which reflects the examiners ability to make a correct positive diagnosis, and values in the range

0.60-0.95 have been reported with a weighted pool average of 0.80 [109]. Additionally, this variability is most pronounced for the type of PMDs detected in this study with larger oral cancers more easily diagnosed [110]. The suggestion is that inter-examiner variability in this population could be reduced by utilising dentists with more specific experience of managing patients with paan-related mucositis. Ideally, the levels of inter-examiner variability need to be assessed quantitatively by a protocol whereby all dentists assess the same patient independently in a blinded manner.

2-4.06 Screening Site and Screening Effectiveness

There was considerable difference in the proportion of referrals coming from each screening site. The screening session on the Isle of Dogs proved very popular with 68 individuals being screened however there were no referrals and it was noted that there were relatively fewer high-risk individuals assessed in comparison to other sites such as the East London Mosque and Watney Market. These latter sites are in the areas of highest Bangladeshi population whilst the Isle of Dogs was included at the request of the CAG in the interest of equality (Figure 2-06). Whilst on site at the Isle of Dogs the mobile dental unit was parked in a supermarket car park and the screening team were routinely approached by families and younger people requesting a screening with proportionally fewer high-risk individuals to be actively targeted. In comparison, the East London Mosque site is just outside one the UK's largest Mosque's where over 5000 worshippers can be simultaneously accommodated for congregation and prayers. The Brady Arts Centre site was similarly situated in an area if high Bangladeshi population density and in close proximity to another Bangladeshi centre of worship. Other

sites at which proportionally more individuals were referred included Bethnal Green Road and Watney Market both busy high street shopping districts and again in the wards of Tower Hamlets with highest Bangladeshi population density. From this it can be suggested that the most productive sites for screening were both in the areas of highest Bangladeshi population and in close proximity to regularly utilised amenities such as worship sites and shopping facilities. At other sites significant numbers of individuals maybe screened but relatively few will be high-risk. At these sites the unquantifiable secondary benefits of increasing awareness of oral cancer, that maybe associated with a community based screening programme, are likely to be the main benefit.

2-4.07 Minimal Data Collection

Minimal data collection on the individuals screened was attempted unless referral was indicated. This was a consensus decision of the CAG as it was felt that people were more likely to attend an anonymous ‘drop-in’ service and the flow of patients would be quicker thereby removing some potential barriers to access for the Bangladeshi population. Preliminary data suggested that each patient could be screened in an average of 3 minutes but referrals would take significantly longer whilst the dentist informed and reassured the patient and answered any questions in order to minimise anxiety. On the busiest session, with an average 6 hours of active screening, 82 patients were screened indicating each screening lasted approximately 4 minutes taking into account that on this occasion 2 patients were referred. Experience from the screening sessions indicated that at busy sites patients were kept waiting for considerably longer than four minutes but the bilingual advocates attempted to either convince high-risk individuals to wait or

return at other times on the same day. If asked to attend other sessions they were also asked to bring along any high-risk friends or relatives who may also wish to be screened. It was not possible to quantify how many patients re-attended or even if patients were screened on more than one occasion due to the paucity of data collected.

As no individuals refused to answer questions about their risk habits to the dentist when assessing risk profile it would appear feasible to collect this data without interfering with the consultation time or discouraging patients. The dentist could also complete a simple mouth map of oral mucosal lesions observed, whether requiring referral or not. This would provide evidence of the proportion of individuals with mucosal abnormality screened at each session and allow for inter-examiner variability quantification.

2.5.01 CONCLUSION

The screening outcomes we achieved suggest that a community based screening approach is viable within the Tower Hamlets population. Analysing the soft gold standards of (a) numbers of positive screened individuals and (b) numbers of PMDs diagnosed suggests that this protocol is comparable to other programmes in the published literature. Because of the predominantly South Asian population screened, the outcomes are better modelled against screening programmes from the Asian subcontinent due to their specific cultural and lifestyle risk factors for OSCC. The screening protocol could be improved by more extensive data collection without creating barriers to access and by focussing on sites of highest footfall in the areas of highest proportion Bangladeshi population.

CHAPTER THREE

EVALUATION OF A TARGETED HIGH-RISK SCREENING PROGRAMME FOR ORAL CANCER IN TOWER HAMLETS AND NEWHAM

Chapter 3

Evaluation of a Targeted High-Risk Screening Programme for Oral Cancer in Tower Hamlets and Newham

3-1.01 INTRODUCTION

Evaluation of the outcomes of the ten session pilot screening programme in Tower Hamlets during 2006 was encouraging. In addition to the 4 PMDs detected in high-risk individuals there was some indication that screening in the community accessed individuals that would not conventionally utilise healthcare services. This was most directly from asking those that initially failed to attend their referral appointment at the secondary care centre but were eventually convinced to do so. The fact that six positively screened individuals never attended the hospital may also indicate some barriers to access for this service that the mobile dental screening was able to overcome. In addition, the oral cancer awareness programme in conjunction with the screening sessions was very well accepted as reported by community representatives in the CAG. In particular, they commented on the targeting of lifestyle factors specific to the Tower Hamlets Bangladeshi population e.g. paan supari, in a manner comprehensible to this population as paan usage is an integral ritual, tradition and culture of Bangladeshi society. This is consistent with the available data on minority ethnic group's utilisation of cancer services where direct methods of user involvement are preferred [111].

Evaluation of the screening protocol revealed several areas where development could significantly improve the programme such as data collection, the referral

process and screening site allocation. Therefore, an optimised ‘Phase II’ screening protocol was developed with the CAG stakeholders and additional funding allocated for implementation.

3-2.01 DEVELOPMENT OF AN ENHANCED SCREENING PROTOCOL

3-2.02 Identification of Phase II Screening Sites in Tower Hamlets

Twenty four further screening sessions were planned in Tower Hamlets. The original ten screening sessions were fairly evenly divided between the wards in Tower Hamlets, with exact locations of the mobile dental unit based on advice from the CAG using census data and local knowledge.

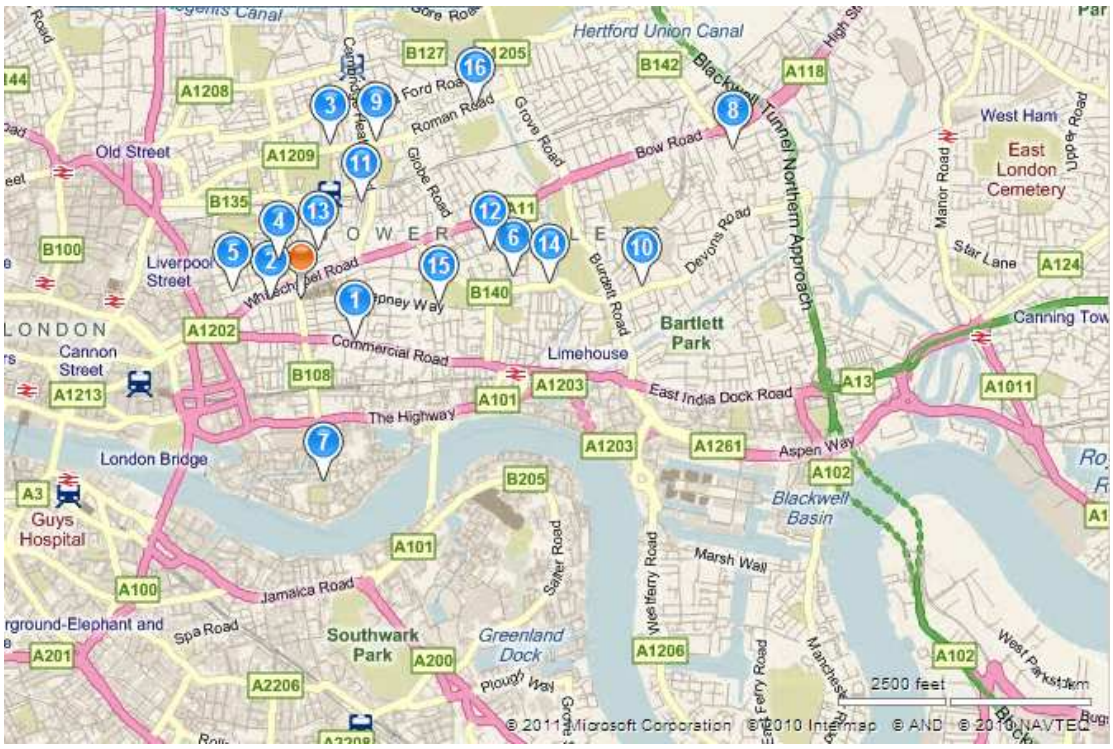


Figure 3-01: Phase II screening sites in Tower Hamlets. (1) Watney Market, (2) East London Mosque, (3) Bethnal Green Road, (4) Brady Arts Centre, (5) Spitalfields Practice, (6) Stroudley Walk, (7) Shahajalal Mosque, (8) Wapping Bangladeshi Association, (9) Bethnal Green Library, (10) Stebon Primary School, (11) Collingwood Estate One, (12) Aden House Womens Association, (13) Whitechapel Sports Centre, (14) Lifra Hall, (15) Redcoat Community Centre, (16) Globe Town Market. (Map adapted from www.bing.com.)

Evaluation of the outcomes of screening suggested that the most productive sites were in the areas of highest Bangladeshi population and in close proximity to regularly utilised amenities such as worship and shopping facilities. Phase II

screening sites were chosen, in conjunction with the CAG, with this information in mind and less emphasis on distributing screening sites evenly around the borough. Figure 3-01 shows the new locations clustered around the most productive previous sites of Watney Market, East London Mosque, Bethnal Green Road and Brady Arts Centre with their proximity to local facilities and travel routes within the areas of highest Bangladeshi population. The Asda Isle of Dogs site was abandoned (Figure 2-09) and not replaced with a new venue in the proximity. A new location at Shahajalal Mosque in the St Katharine's and Wapping ward was chosen because of the activity of the local Bangladeshi association as a meeting point for elders. Unfortunately, it was not possible to use the Mile End Park site due to temporary parking restrictions for the Mobile Dental Unit during the planned screening activity time so a nearby alternative was chosen at Globe Town Market to target the North Eastern wards of Tower Hamlets.

3-2.03 Screening in Newham

In addition to Phase II screening sessions in the borough of Tower Hamlets, five sessions were to be carried out in the neighbouring borough of Newham (Figure 3-02) because of the known similarities in cultural and socioeconomic risk factors for oral cancer amongst the Newham population. Figure 2-03 shows the high proportion of Bangladeshi's in the population of specific areas of Newham, as occurs in Tower Hamlets. However, there are significant differences in the ethnic mix in comparison to Tower Hamlets because Newham also has high proportions of Indian and Pakistani individuals (Figure 2-04).

Figure 3-02: The London boroughs showing the adjacent position of Newham to Tower Hamlets. (Adapted from www.guardian.co.uk)

Newham is also the 6th most socio-economically deprived borough in England [54]. Therefore, whilst Tower Hamlets provides a relatively homogenous Bangladeshi population in which to target OSCC screening activity, Newham potentially allows evaluation of the strategy in a more diverse South Asian population. The local advertising campaign and oral cancer awareness programme was conducted in Punjabi and Hindi as well as Sylheti to target the Indian and Pakistani populations in addition to the Bangladeshi. Punjabi is the most commonly understood language of the Pakistani population and Hindi for the Indian population of the UK [54]. The link workers recruited specifically for the Newham project were fortunately, and as is common amongst many speakers of these languages, able to converse in both Punjabi and Hindi.

3-2.04 Identification of Screening Sites in Newham

Five sessions were planned in the borough of Newham and a separate CAG was convened, again comprising local stakeholders from the PCT, Smoking Cessation and Dental Access teams, Barts and The London Cancer Services, Oral Medicine and Oral and Maxillofacial Surgery teams, community organisations and patient representatives. It was determined the most appropriate locations in the borough centred on the Green Street shopping precinct due to the high volume of passing individuals, proximity to travel services and the ability to station a mobile dental unit. The locations at the north, central and south ends of Green Street market are shown in Figure 3-03.

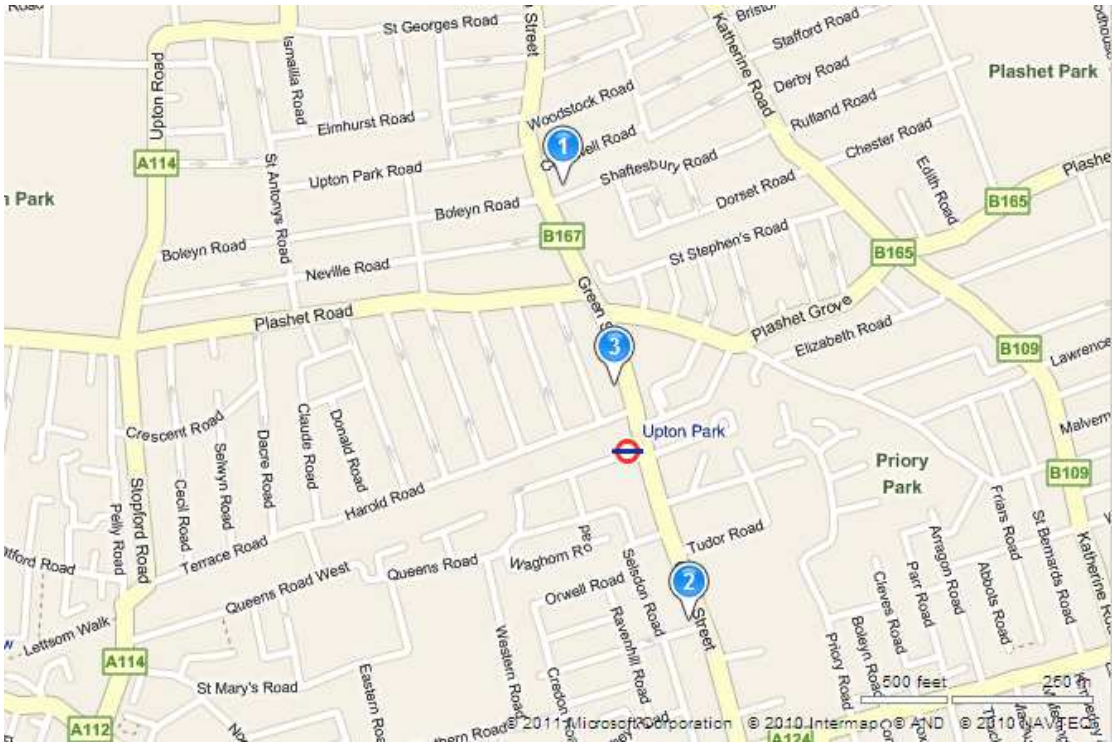


Figure 3-03: The sites in Newham chosen for screening activity. (1) Shaftesbury Road, (2) Green Street (South), (3) Green Street Market. (Map adapted from www.bing.com).

3-2.05 Roles of the Bilingual Advocate

The most concerning issue from the 2006 screening activity in Tower Hamlets was the non-compliance of six (out of 20) individuals screened positive with referral to the secondary care centre for definitive diagnosis. The bilingual advocate's primary role was to provide language support during screening sessions and to follow up the referral process. In Phase II, the advocates had a more integral role in the delivery process, before, during and after the screening sessions. They were more involved in actively recruiting participants to the oral cancer screening sessions and onto tobacco cessation services. Advocates attended screening sites in advance to advise local people of the upcoming screening activity particularly in local community associations. Advocates further facilitated the referral process and kept in contact with individual patients including attending outpatient appointments at the secondary referral centre. The intention was to eliminate drop-out of positively screened individuals before definitive diagnosis at the secondary referral centre. The time-lag for a referral appointment was less than 2 weeks because of specific capacity created on the Oral Medicine clinics for positively screened individuals from each session.

3-2.06 Dentist Calibration

Despite the small sample size, there was apparent variability in the numbers of screened patients being referred by each dentist confirmed by their expressions of difficulty in determining which lesions to refer. The literature suggests that sensitivity and specificity of visual screening for OSCC is unaffected by who is undertaking the screening [109] and although it would be disingenuous to calculate sensitivity and specificity values from our results, even with the soft gold

standards, this was not our qualitative experience. This maybe due to the specificities of the Tower Hamlets population with the difficulties of assessing paan users who routinely have significant paan associated mucositis and red-brown staining of the mucosa with whiter hyperkeratotic areas (Figure 3-04).

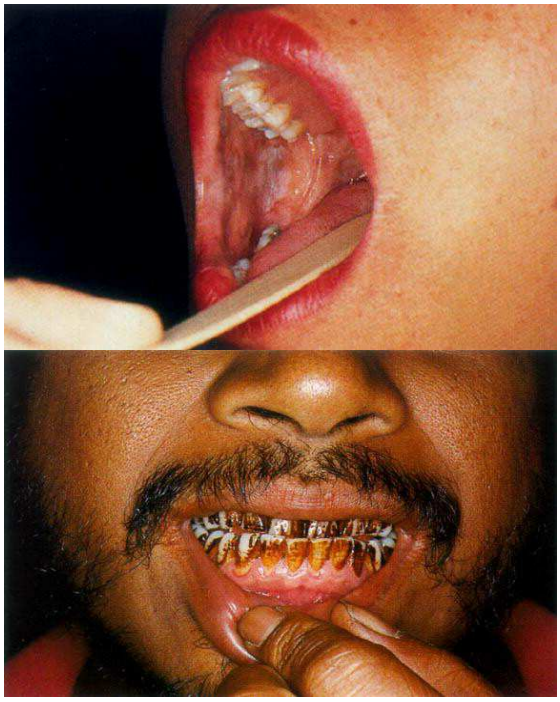


Figure 3-04: The reddish-brown staining and whiter keratotic areas of buccal mucosa and teeth associated with paan usage, characteristic of the Tower Hamlets population. (From: Norton (1998) [14])

Within our small sample, dentists with more experience of managing these oral mucosal lesions (OMLs) were less likely to refer screened individuals and upon referral the patient was more likely to need a biopsy. Studies around the world have repeatedly shown that dentist's knowledge of oral cancer is inconsistent suggesting their discriminative ability in assessment of OMLs maybe variable even if detection of OSCC is more consistent [112-115]. This would suggest that inter-examiner variability could be reduced by utilising fewer dentists and clinicians with

more specific experience of managing patients with paan-related staining. Therefore for Phase II, two dentists with specific interest and experience of managing patients with oral mucosal disease were recruited. A more robust standardisation and calibration process was developed utilising the WHO 'blue book' Pathology and Genetics of Tumours of the Head and Neck [21]. It was agreed that the fast track referral was only suitable for lesions requiring urgent biopsy i.e. frank OSCC or erythroplakia or non-healing ulcers at a high risk site. Other common oral mucosal findings such paan-associated-mucositis or hyperkeratosis would not be referred unless there was another specific lesion of concern. These patient's would however be advised to reduce their risk factors and seek regular check-ups at their own dentist and especially if symptoms developed. If required they were to be referred via a routine referral route onto the Oral Medicine clinics.

3-2.07 Quantification of Inter-Examiner Variability

To quantify levels of inter-examiner variability during screening, DMFT was used as a suitably quick and objective oral assessment which can be completed independently in a blinded manner on the same patient during the same screening session. DMFT describes the prevalence of dental caries in an individual and is obtained by calculating the number of Decayed (D), Missing (M) and Filled (F) Teeth (T) in an individual. This is an objective measure of dental disease with well defined diagnostic criteria that have been used by over 130 health administrations throughout the world and has a proven capacity to yield reliable, useful, and internationally comparable data on oral health status [116]. Based on experience of dentistry in this population and WHO best practice recommendations the DMFT diagnostic criteria utilised in this study were:

- Maximum DMFT is 32 as all four wisdom teeth are included in the charting to prevent inconsistency due to the common scenario of lost first and second molars leaving just the third molars which may then be mis-charted as second molars.
- If a tooth has both a caries lesion and a filling it is calculated as D only.
- Incipient (non-cavitated) carious lesions were not charted as D.

All patients seen in 3 one hour blocks at three separate screening sessions were independently assessed by both screening dentists for DMFT. As this does not give any reliable information on the clinician's ability to assess for OSCC the presence of OMLs was also recorded in a blinded manner for these patients.

3-2.08 Patient Information Collection

From the 2006 screening programme it was determined that patient data collection was unlikely to prevent or discourage individuals from attending for screening. More extensive data collection would primarily aid in assessing the accuracy of screening activity in targeting high-risk individuals. The biggest risk appeared to be in extending the time taken for each individual screening as the busiest sessions already resulted in patients waiting or being asked to return at another session.

Demographic details were to be collected from all patients screened if they were willing to give this information. Screening would not be denied if a participant did not wish to give their details. Patient details necessary to provide evidence of high risk status were: age, nationality, gender and risk habits (tobacco or areca nut consumption or alcohol usage). This is unlikely to add to screening session time because this information would routinely be obtained verbally as part of the OSCC risk profile determination by the dentist during the screening. In the interests of

brevity, information on other factors potentially linked to OSCC, such as education level and socioeconomic status, which would require validated questionnaires to elucidate accurately, were not attempted by the screening dentists. Additionally, no specific attempt was to be made to determine the constituents of paan i.e. whether tobacco was included, unless referring a screen positive individual. Similarly with smoking, quantification would not be required (numbers of pack years, ounces of tobacco etc) for every patient.

Experience from screening in Tower Hamlets indicated that a significant proportion of people examined had pre-existing OMLs, although not necessarily PMDs. The clinical diagnosis, or description if diagnosis was unknown, of all OMLs was to be recorded whether referred or not. This was facilitated by developing a list of clinical diagnosis agreed between the screening dentists and reviewed as necessary throughout the programme. OSCC predominantly develops at specific sites in the mouth suggesting that OMLs at different sites carry different risks of malignant conversion, therefore the site of lesions was recorded on a mouth map to determine the proportion of OMLs at high risk sites. Chapter 4 provides more specific details of OML recording in the screened population.

Data collection was approved by The East London and the City Research Ethics Committee and the Joint Research and Development Office of Bart's and The London (REC 08/H0701/98). Data analysis utilised Excel 2003 (Microsoft, USA) with the StatPro add-on (Kelley School of Business, Indiana University) and STATA 10 (StataCorp LP, Texas, USA). To allow for anonymity all participants initially gave verbal and implied consent to take part in screening but written

consent was obtained for recording of patient related data for all screened patients, if they agreed for data to be recorded. Refusing to participate in the data collection exercise did not prevent patients from being screened or referred. No attempt was made to elicit why participants may not wish to allow recording of their data.

3-2.09 Inclusion Criteria

The criteria used successfully in 2006 were implemented again, where individuals were actively recruited by link workers based on the proposed risk factors for oral cancer in South Asian populations:

- Age 35 years or over
- South Asian ethnic origin
- Practising one or more of the risk behaviours of smoking or chewing tobacco, chewing areca nut compounds (paan) or alcohol usage.

Individuals falling outside these inclusion criteria were not actively recruited by the link workers but were not excluded from screening if they wished to attend.

3-3.01 RESULTS

In 2008 the Phase II oral cancer screening programme, targeting high-risk individuals in the Bangladeshi community of Tower Hamlets, was commenced. A total of 24 screening sessions were undertaken and each session was approximately seven hours in duration and undertaken between 0930 and 1630 with flexible break times dependent on the flow of patients. A similar protocol was undertaken in the neighbouring borough of Newham with five screening sessions targeting high-risk individuals in Newham's more diverse South Asian population. These sessions were approximately 8 hours in duration from 0930-1700 due to the extra availability of Newham's mobile dental unit.

3-3.02 Numbers Screened

In total 835 people were screened during the 24 sessions held in Tower Hamlets, an average of 35 per session, with a range from 12 to 47 with the busiest site on Bethnal Green Road, as in the 2006 screening programme (Table 3-01). The five Newham sessions yielded an average of 55 people screened per session and 276 screened in total with a range of 24 to 76 individuals (Table 3-02). Five sessions suffered significant inclement weather which may have directly affected screening numbers, these were:

Green Street (South) in Newham on Friday 21st March,

Spitalfields Practice on Wednesday 9th April,

Wapping Bangladeshi Association on Saturday 19th April

Redcoat Community Centre on Friday 23rd May

Globe Town Market on Monday 26th May

Table 3-01: Numbers screened and referred from each screening session in Tower Hamlets

Screening Session		Location	Screened	Referred	Dentist
2008	Wednesday 26th March	East London Mosque	42	4	AL
	Monday 31st March	Watney Market	41	5	NS
	Wednesday 2nd April	Bethnal Green Road	43	6	AL
	Saturday 5th April	Stroudley Walk Centre	40	3	AL
	Monday 7th April	Spitalfields Practice	37	8	NS
	Wednesday 9th April	Spitalfields Practice	21	3	AL
	Monday 14th April	Shahajalal Mosque	35	3	NS
	Wednesday 16th April	Wapping Bangladeshi Association	34	2	AL
	Saturday 19th April	Wapping Bangladeshi Association	21	0	AL
	Wednesday 23rd April	Bethnal Green Library	36	2	AL
	Friday 25th April	Stebon Primary School	40	1	AL
	Monday 28th April	Brady Arts Centre	47	4	NS
	Wednesday 30th April	Collingwood Estate Club	15	2	AL
	Saturday 3rd May	Aden House Women's Association	12	1	AL
	Monday 5th May	Bethnal Green Road	47	1	NS
	Wednesday 7th May	Whitechapel Sports Centre	46	1	AL
	Monday 12th May	Bethnal Green Road	43	0	NS
	Wednesday 14th May	Lifra Hall	42	2	AL
	Saturday 17th May	East London Mosque	46	1	AL
	Monday 19th May	Watney Market	31	0	NS
	Wednesday 21st May	East London Mosque	41	2	AL
	Friday 23rd May	Redcoat Community Centre	22	1	AL
	Monday 26th May	Globe Town Market	20	1	NS
	Wednesday 28th May	Whitechapel Sports Centre	33	2	AL
		TOTAL 24 SESSIONS	835	55	

Table 3-02: Numbers screened and referred from the screening sessions in Newham.

Screening Session		Location	Screened	Referred	Dentist
2008	Friday 21st March	Green Street (South)	24	1	AL
	Saturday 22nd March	Green Street Market	64	5	AL
	Sunday 30th March	Green Street Market	41	2	NS
	Saturday 26th July	Shaftesbury Road	71	1	AL
	Sunday 27th July	Shaftesbury Road	76	2	AL
		TOTAL 5 SESSIONS	276	11	

3-3.03 Patient Data

Data recording was completed for all 1111 screened participants in Newham and Tower Hamlets. Despite the similarities in screening protocols (inclusion criteria, advertising, staff etc.) the populations screened in Tower Hamlets and Newham markedly differ in aspects of their demographics and more importantly their risk factors for OSCC (Table 3-03). This suggests these differences are predominantly inherent socioeconomic and cultural properties of the populations screened.

Table 3-03: Demographics and risk factors for the screened population compared to the referred population in Tower Hamlets and Newham.(student t test, ^ chi-squared)*

		Tower Hamlets			Newham		
		Screened	Referred		Screened	Referred	
Number of people		835	55		276	11	
Age in years - mean (SD)		42.3 (15.9)	52.0 (14.1)	p<0.05*	38.9 (12.4)	49.9 (15.1)	p<0.05*
Gender mix - M:F		55:45	36:64	p<0.05^	67:33	64:36	p=0.53^
Ethnicity -	Bangladeshi	84%	96%	P<0.05^	27%	64%	P<0.05^
	Other South Asian	7%	0%	P<0.05^	39%	27%	P<0.05^
Risk Factors -	Smoking	44%	40%	p=0.41^	37%	55%	P<0.05^
	Paan (+/- tobacco)	58%	95%	P<0.05^	21%	64%	P<0.05^
	Alcohol	12%	7%	p=0.05^	31%	45%	P<0.05^

3-3.04 Age of the Screened Population

The standard deviation of the mean age for both Tower Hamlets and Newham screened populations, includes the under 35 age group, suggesting a significant proportion screened were younger patients (Table 3-03). It could be accepted that older individuals are less likely to attend a chance screening on a mobile dental unit if access was difficult but some of the screening sites chosen in Tower Hamlets were specifically targeting older people who use day centres, where the staff were actively involved in recruiting those at high-risk. Despite the clear

overlap in age ranges between the screened population in Tower Hamlets and Newham shown by the descriptive standard deviation data the means of the two groups were significantly different (p=0.0052) suggesting that the targeting of older people in Tower Hamlets was effective. The referred sub-populations were also significantly older than the general screened population in both Tower Hamlets and Newham (Table 3-03), which is consistent with age being a known risk factor for OSCC. The Trivandrum Oral Cancer Screening Study [5] reported mean ages of the screened population to be about 49 years, similar to the referred populations here.

3-3.05 Gender of the Screened Population

In Tower Hamlets the male to female ratio was 55:45 and considerably more even than Newham (67:33) (Table 3-03). Some sites in Tower Hamlets were specifically targeting females e.g. Aden House Women's Association and one of the sessions at Spitalfields practice where 100% of those screened were female. Newham screening was on a busy high-street with no specific attempt to target women so the 67% male predominance suggests that in this population, men's uptake of screening services is significantly greater than women's, which is consistent with the literature [97]. However, this effect appears to be effectively compensated for by specifically targeting women in the Tower Hamlets screening protocol. The referred population consisted of significantly more women in the Tower Hamlets group than the screened population whilst the Newham referred and screened population gender mix was equivalent.

3-3.06 Ethnicity of the Screened Population

Census data shows the ethnic make-up of Tower Hamlets and Newham boroughs is substantially different, with a more homogenous South Asian population of predominantly Bangladeshi origin in Tower Hamlets. Whereas, the South Asian population in Newham has a much higher proportion of Indian's and Pakistani's in addition to Bangaldeshi (Figure 2-04). This was evident in the screened populations with Tower hamlets comprising 84% Bangladeshi and just 7% of the other South Asian groups making a total of 91% of South Asian origin. In Newham, just 27% of those screened were Bangladeshi whilst 39% were other South Asian groups, giving 66% in total of South Asian origin (Table 3-03). Location of the mobile unit is likely to be a major cause of this difference as many Tower Hamlets sites were closely associated with community facilities utilised specifically by South Asian populations e.g. the East London Mosque. In contrast, the Newham screening sites were all in the borough's main shopping precinct and therefore the screened population would be more likely to represent the overall population. The referred population was again significantly different to the screened people in both Tower Hamlets and Newham with a higher proportion of Bangladeshi individuals referred than screened.

3-3.07 Reported Risk Factors

Each screened patient was specifically asked if they indulged in any of three risk factors for OSCC which were smoking, paan usage and alcohol consumption. The finding that 58% of the Tower Hamlets group use paan (Table 3-03) indicates the effectiveness of specifically targeting this clearly visible risk factor in this population where the prevalence of paan usage has been variably reported as

between 13 to 48.5% [88, 93]. However, just 21% of the Newham cohort were paan users, presumably related again to the screening locations. Approximately, two fifths of both populations were smokers but alcohol use was reportedly much higher in the Newham group (31% compared to 12%) indicative of the ethnic mix where alcohol is not a significant risk factors amongst the Bangladeshi population specifically [88]. Overall the demographic and risk factor descriptive data would suggest the screening activity was extremely successful at targeting the high-risk paan using population in Tower Hamlets. This is reinforced by 95% of referred individuals using paan, which is significantly higher than the general screened population in Tower Hamlets although the levels of alcohol and smoking in the two groups were equivalent. In contrast, the Newham referred population were significantly more likely to use both paan and alcohol as well as smoke than the whole Newham screened population.

3-3.08 Referrals to Secondary Care Services

Of the 835 people screened in Tower Hamlets 55 (6.6%) were referred with suspected cancer for further investigation (Table 3-01) whilst 11 (4.0%) of 276 were screened positive in the Newham campaign (Table 3-02).

3-3.09 Referrals by Screening Site

In Tower Hamlets all of the screening sites yielded referrals so although there were 3 sessions at which no individuals were screened positive, other sessions at the same site produced at least one referral (Table 3-01).

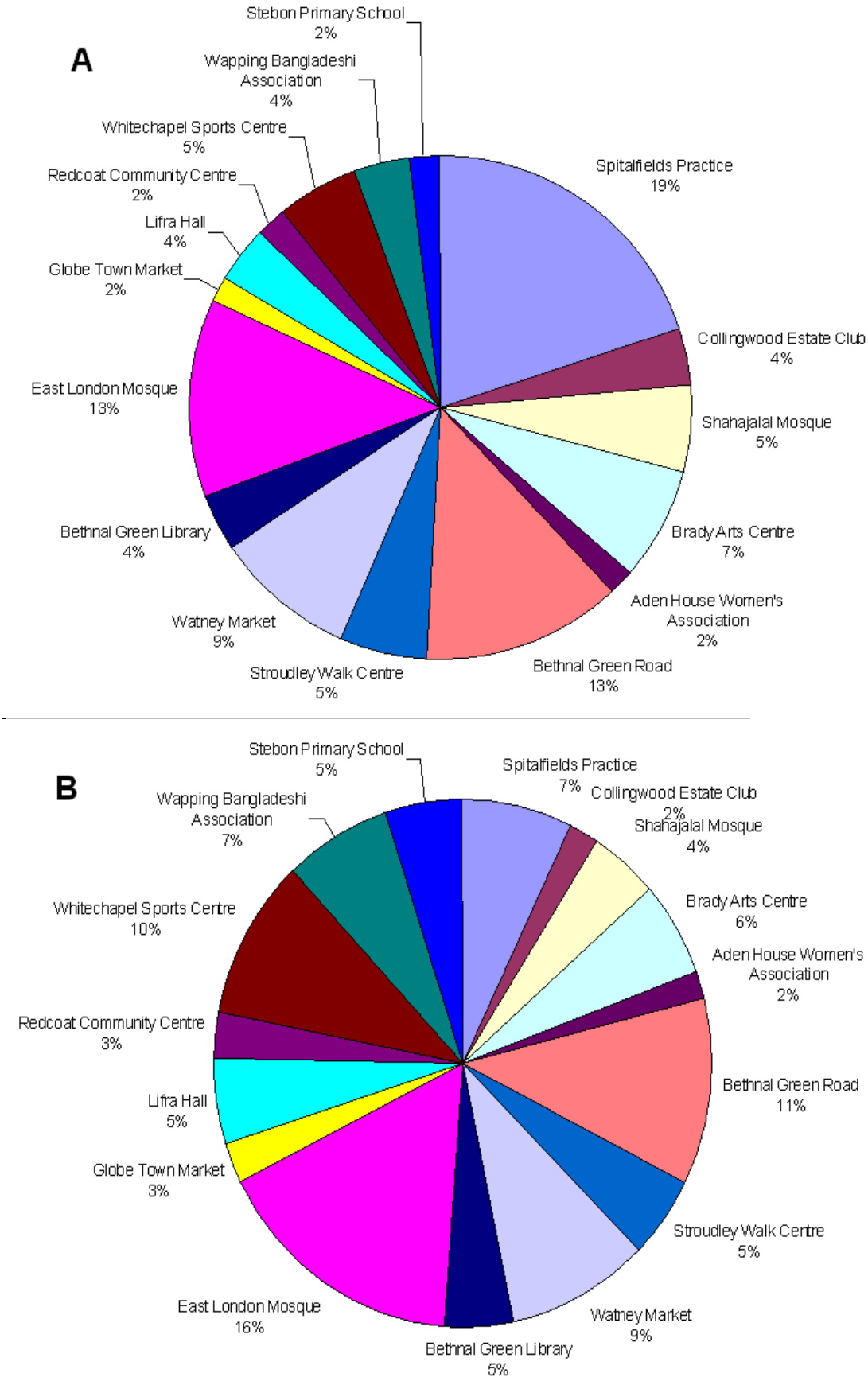


Figure 3-05: (A) The proportion of referred patients from each screening site in Tower Hamlets and (B) the proportion of patient examinations by screening location. (Total screened = 835, Total referred = 55).

At Spitalfields Practice, 58 people were screened in 2 sessions and 11 (19%) referred. This equates to just 7% of all screened patients being seen at this location, yet 19% of all referrals originated from here. It was noted that the adjacent general medical practice had actively recruited patients to be screened following publicity in the preceding weeks. As in the 2006 screening programme East London Mosque and Bethnal Green Road were sites contributing significant proportions of all referrals (13% each) and were also sites at which substantial proportions of all screened individuals were seen (16% and 11% respectively). The other sites revisited from 2006 were Brady Arts Centre and Watney Market which produced 7% and 9% of referrals from 6% and 9% of all screenings, respectively (Figure 3-05).

All three sites in Newham also yielded screen positive individuals for referral (Table 3-02). The majority of referrals (64%) were from the two sessions on Green Street Market but only 38% of all screenings were at this site, whilst Shaftesbury Road accounted for 53% of screenings but only 27% of referrals (Figure 3-06).

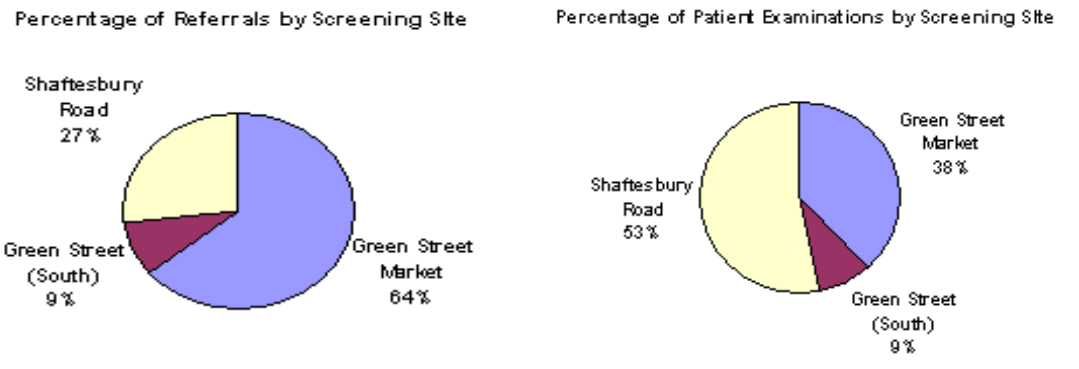


Figure 3-06: Proportion of individuals screened at each site in Newham (Total screenings 276) and percentage of referrals by screening site (Total 11 referrals).

3-3.10 Referrals by Dentist

In total Dentist AL screened 534 individuals at 16 sessions in Tower Hamlets and referred 33 (6%) whilst Dentist NS screened 301 at 8 sessions, referring 22 (7%). On the Newham screening programme Dentist AL screened 235 patients at 4 sessions, referring 9 (4%) whilst Dentist NS screened 41 and referred 2 (5%) suggesting a good degree of consistency between both examiners (Figure 3-07).

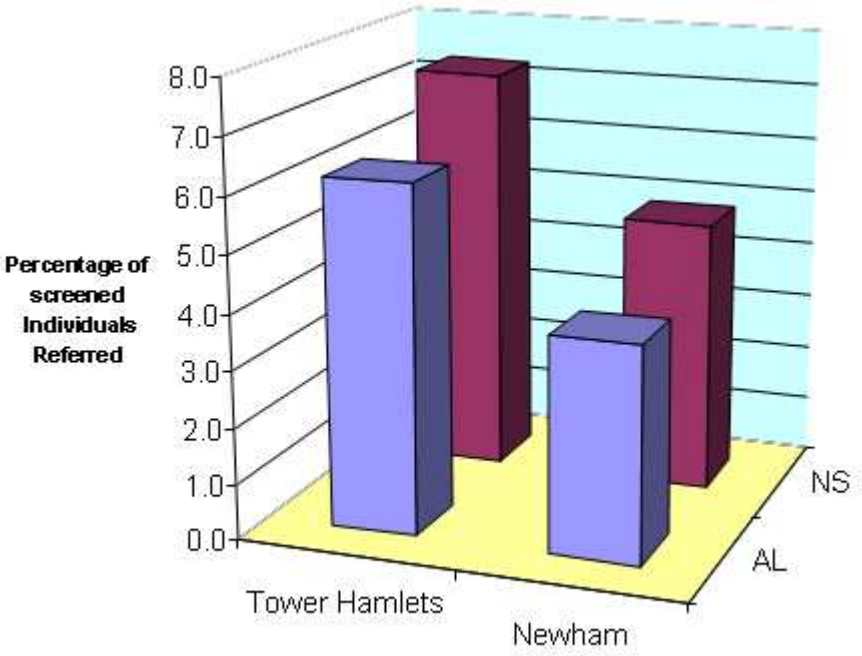


Figure 3-07: Percentage of screened patients referred on the Tower Hamlets and Newham screening programme by Dentist AL and NS. (Total 66 referrals).

3-3.11 Inter-Examiner Variability

17 patients were independently assessed by both examiners in series for OMLs and DMFT scores (Table 3-04). All patients included were at least partially dentate and intra-examiner agreement on DMFT was very high indicated by a correlation value of 0.97 (Figure 3-08) although the limitations of DMFT assessment in indicating ability to detect oral cancer by direct visual examination are accepted.

*Table 3-04: Pearson correlation for 17 independently screened patients assessed for DMFT and the presence of OMLs. ***** indicates the patient screened positive for OSCC and referred by both clinicians.*

	Patient	DMFT score		OML detected	
		Examiner NS	Examiner AL	Examiner NS	Examiner AL
Session 1	NS310301	14	17	-	-
	NS310302	4	5	paan mucositis	paan staining
	NS310303	26	23	-	-
	NS310304*	4	4	**leukoplakia + paan staining**	**erythroleukoplakia + paan staining**
	NS310305	0	0	-	-
Session 2	NS070425	5	6	-	-
	NS070426	4	3	-	-
	NS070427	3	3	periapical abscess UL5	-
	NS070428	9	7	traumatic ulcer	traumatic ulcer
	NS070429	0	0	-	-
Session 3	NS050504	4	3	periodontal abscess	abscess + sinus tract
	NS050505	17	21	-	-
	NS050506	3	3	-	-
	NS050507	0	0	frictional keratosis	frictional keratosis
	NS050508	3	2	median rhomboid glossitis	median rhomboid glossitis
	NS050509	19	16	-	-
	NS050510	7	8	-	-
Average		7.2	7.1	7 lesions	6 lesions
Correlation		0.97		0.88	

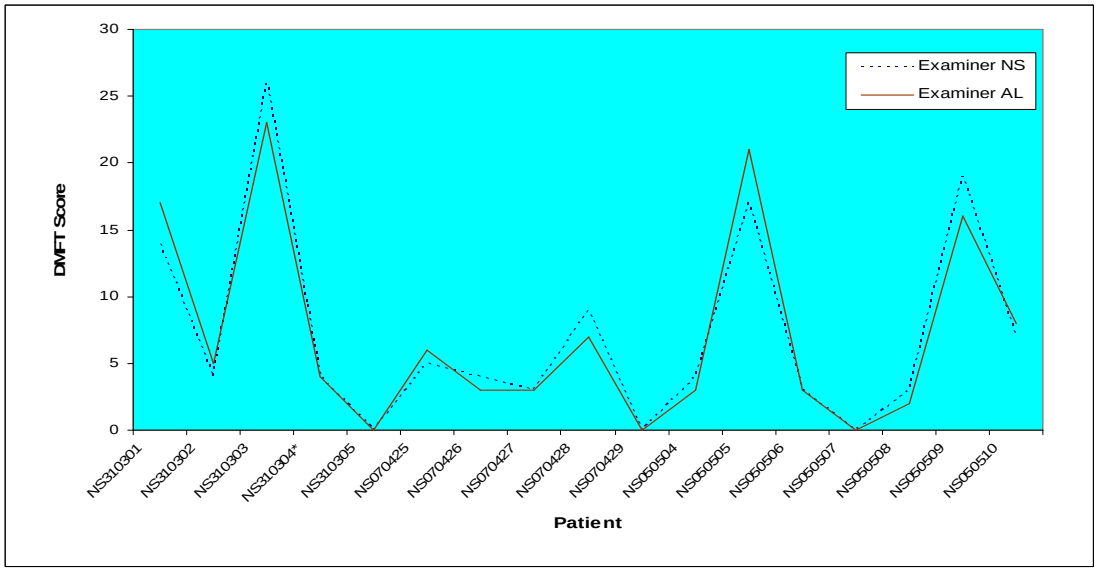


Figure 3-08: Shows the correlation (r = 0.97) between examiners for DMFT scores.

There was however good correlation for detection of OMLs with a correlation of 0.88 for lesions detected, however only seven lesions were evident in the 17 patients. Dentist AL detected 6 lesions and the extra one picked up by dentist NS was identified as a periapical abscess of an upper left second premolar although it was not possible to determine if this was associated with a sinus tract or papillae at time of assessment. Upon further discussion the two examiners decided that if no sinus tract was evident, dentoalveolar pathology would not be included. On a later screening calibration session another dental (periodontal) abscess with draining sinus tract was noted by both clinicians indicating agreement on this issue. Further evidence for these dentists level of agreement in detection and assessment of OMLs is the one referral by both examiners, independently reported as a clinical diagnosis of “erythroleukoplakia” and “leukoplakia in paan staining”. The histological diagnosis remains unavailable as this patient failed to attend the secondary referral centre despite exhaustive attempts to contact them.

3-3.12 Outcomes of Referrals to Secondary Care Services

Despite the direct involvement of the culturally appropriate advocates in chasing referrals and offering to attend appointments with the positively screened individuals, 14 patients (25%) of the 55 referred from Tower Hamlets and one (9%) of the 11 referred from Newham screening sessions failed to attend the secondary referral centre for definitive diagnosis (Table 3-05 and Table 3-06). As previously, all feasible attempts were made to make contact with these individuals by both telephone and post and home visit. Additionally, two patients attended for their initial Oral Medicine consultant clinic appointment, where they were advised a

biopsy was required, but then failed to attend for the biopsy itself despite repeated attempts to contact them.

*Table 3-05: Definitive primary diagnosis and outcome after referral to secondary care services of positively screened individuals from Newham. (DNA= did not attend secondary referral centre; *****= definitive diagnosis confirmed by histology; #= did not return for biopsy after initial diagnosis at consultant clinic). Four diagnosed with potentially malignant disorders are highlighted.*

Newham (Total Referrals 11)		
Patient	Primary Diagnosis	Outcome
AL210307	hyperkeratosis*	discharge
AL220304	**oral submucous fibrosis***	review
AL220308	DNA	-
AL220317	hyperkeratosis*	review
AL220331	hyperkeratosis*	review
AL220363	hyperkeratosis*	discharge
NS300302	**oral submucous fibrosis***	review
NS300333	candidiasis	review#
AL260712	**mild/moderate dysplasia***	review
AL270754	**lichenoid***	review
AL270771	hyperkeratosis*	discharge

*Table 3-06: Definitive primary diagnosis and outcome after referral to secondary care services of positively screened individuals from Tower hamlets. (DNA= did not attend secondary referral centre; *****= definitive diagnosis confirmed by histology; [#]= did not return for biopsy after initial diagnosis at consultant clinic). 13 diagnosed with potentially malignant disorders are highlighted.*

Tower Hamlets (Total Referrals 55)		
Patient	Primary Diagnosis	Outcome
AL260314	hyperkeratosis*	review
AL260337	DNA	-
AL260339	**oral submucous fibrosis***	review
AL260342	hyperkeratosis*	review
NS310304	DNA	-
NS310334	DNA	-
NS310331	**mild/moderate dysplasia***	review
NS310330	**lichen planus***	review
NS310339	hyperkeratosis*	review
AL020409	DNA	-
AL020411	hyperkeratosis	review[#]
AL020430	DNA	-
AL020439	hyperkeratosis*	discharge
AL020441	**oral submucous fibrosis***	review
AL020443	haemangioma	discharge
AL050408	DNA	-
AL050423	hyperkeratosis*	review
AL050431	hyperkeratosis*	discharge
NS070401	fibro-epithelial polyp*	discharge
NS070405	DNA	-
NS070415	hyperkeratosis*	review
NS070417	DNA	-
NS070422	hyperkeratosis*	review
NS070422	**lichenoid***	review
NS070423	**lichenoid***	review
NS070434	hyperkeratosis*	review
AL090411	**mild/moderate dysplasia***	review
AL090419	hyperkeratosis*	discharge
AL090421	candidiasis*	review
NS140418	**mild/moderate dysplasia***	review
NS140429	DNA	-
NS140434	DNA	-
AL160402	**mild/moderate dysplasia***	review
AL160427	**severe dysplasia***	review
AL230418	physiological pigmentation	discharge
AL230426	DNA	-
NS280407	hyperkeratosis*	review
NS280412	Hyperkeratosis*	review
NS280422	hyperkeratosis*	review
NS280436	haemangioma	discharge
AL250403	hyperkeratosis	review
AL300401	hyperkeratosis*	review
AL300413	hyperkeratosis*	discharge
AL030505	DNA	-
NS050503	hyperkeratosis*	review
AL070519	**oral submucous fibrosis***	review
AL140528	hyperkeratosis*	review
AL140539	hyperkeratosis*	review
AL170545	DNA	-
AL210504	physiological pigmentation	discharge
AL210516	hyperkeratosis*	discharge
AL230522	**oral submucous fibrosis***	review
NS260516	DNA	-
AL280512	**mild/moderate dysplasia***	review
AL280531	hyperkeratosis*	discharge

3-3.13 Definitive Diagnosis

The screening protocols and examiners were the same for both Tower Hamlets and Newham programmes and resulted in a relatively small number of referrals, therefore the outcomes are presented together. Figure 3-09 shows the definitive diagnosis for the 51 attendees at the secondary referral centre from both Tower Hamlets and Newham screening sessions.

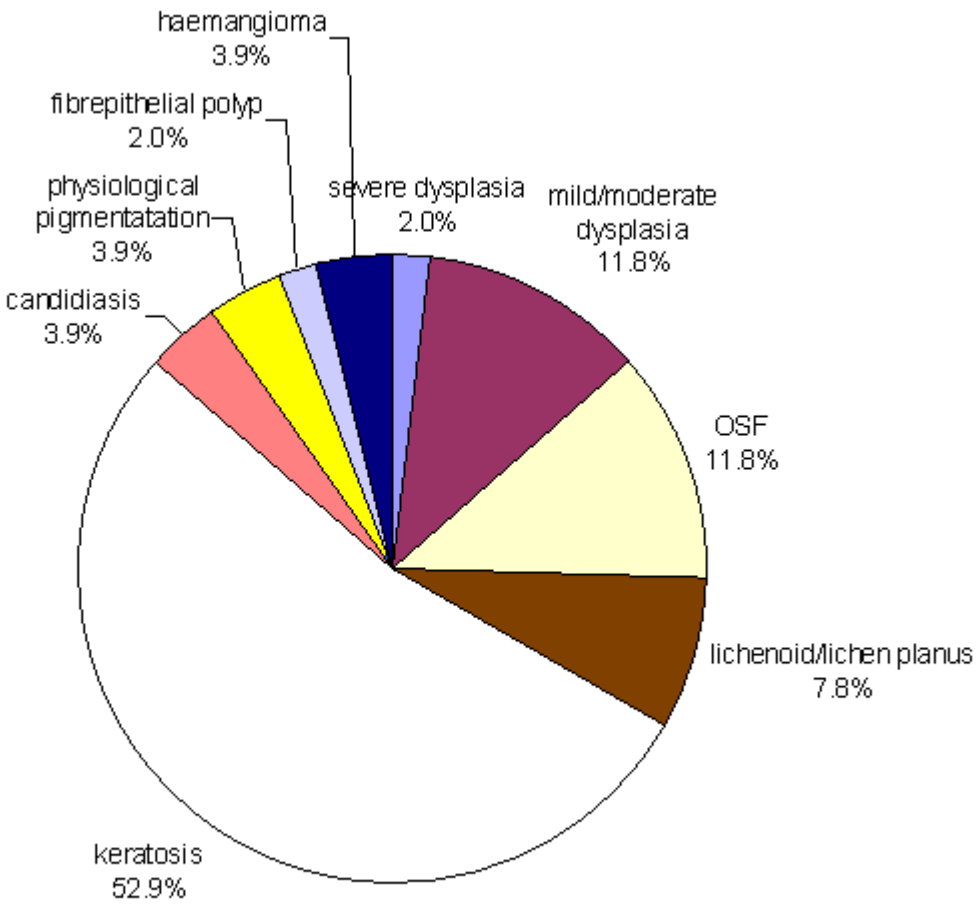


Figure 3-09: Definitive diagnosis for 51 positively screened patients attending the secondary care centre.

3-3.14 Potentially Malignant Disorders

As with the screening programme in 2006 there were no frank oral cancers detected. Seven patients were diagnosed with dysplastic lesions, one of which was severe dysplasia with the highest probability of OSCC, hence it was completely excised. One moderately dysplastic lesion was superinfected with candidal hyphae, which was treated with repeat courses of antifungals until a third biopsy, taken some months later, showed only mild focal dysplasia. All patients diagnosed with dysplastic lesions remain under regular review and were enrolled into a tobacco cessation programme to help manage their risk factors for oral cancer. Many patients presented with more than one OML and therefore the primary diagnosis from the Oral Medicine specialist is presented e.g. a patient referred for a paan-associated leukoplakia diagnosed histologically as dysplastic who also had lichen planus and at the time of assessment a discharging sinus tract from a dental abscess, would only be reported as dysplasia.

Six referred patients were diagnosed with the potentially malignant mucosal disorder OSF and remain under review for evidence of malignant change as well as being helped to reduce tobacco and particularly paan usage. Four others were diagnosed with lichen planus or lichenoid type mucosal lesions, which in view of their associated risk factors of tobacco and areca nut consumption, must also be regarded as potentially malignant. With the seven dysplastic lesions a total of 17 out of 51 (33%) of the positive screened patients were diagnosed with PMDs (Figure 3-10).

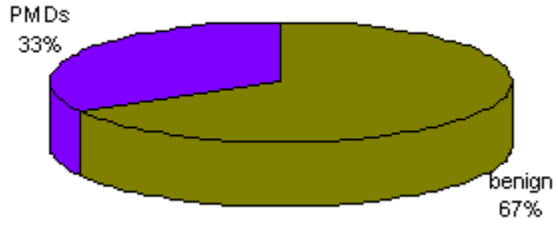


Figure 3-10: Potentially malignant disorders (PMDs) and benign lesions within the positively screened population who attended for biopsy (Total 51).

3-3.15 Histological Diagnosis

Of the 51 positively screened patients who attended the secondary referral centre, 46 (90%) required scalpel biopsy and histological examination. The other 5 were diagnosed clinically by the Oral Medicine specialist as benign lesions with no indication for histological evaluation (Figure 3-11). Two scheduled for biopsy never attended the hospital to have the procedure completed.

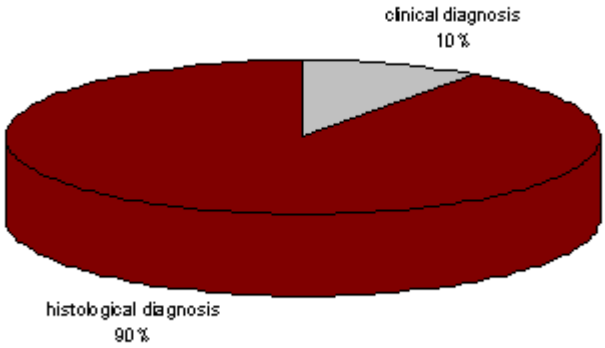


Figure 3-11: Method of achieving the definitive diagnosis for screen positive individuals at the tertiary referral centre. (Total 14)

3-3.16 Benign Lesion Management

The majority of referred patients were diagnosed with benign lesions (Figure 3-10). Hyperkeratosis without evidence of dysplasia was diagnosed in 52.9% (Figure 3-09). Despite lacking evidence of malignancy or dysplasia only a small proportion of the patients diagnosed with benign hyperkeratosis were in a position to be discharged i.e. no significant risk of developing PMDs or having access to appropriate primary care services to monitor their OMLs. For this reason, 73% of all referred patients remain under review in the Oral Medicine department 1 year after the last screening session (Figure 3-12). Two patients presented with benign lesions diagnosed as physiological pigmentation who were discharged to the care of their General Dental Practitioner with appropriate advice on the management of risk factors for oral cancer. Also discharged were two patients presenting with small haemangiomas which did not require surgical intervention. Another patient discharged from further follow-up, after histological evaluation, was shown to have an ulcerated fibro-epithelial polyp.

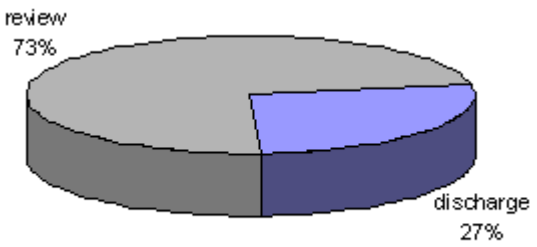


Figure 3-12: Outcome of referral for the positively screened population who attended the secondary referral centre at 1 year after the last screening session. (Total 51).

3-4.01 DISCUSSION

In total 835 people were screened in Tower Hamlets and 55 referred (6.6%), whilst 276 were screened in Newham and 11 referred (4.0%). These referral rates are broadly comparable with the 2006 screening campaign (4.1%) and that reported in previous oral cancer screening initiatives [62, 109]. In particular, this is of the same order of magnitude as the 5.9% referral rate reported in the Trivandrum Oral Cancer Screening Study [5]. This would suggest the screening protocol overall is comparable to other programmes in the published literature and supports the notion that customising a delivery model to a South Asian population, wherever it maybe located, is both effective and feasible.

3-4.02 Evaluation of Clinical Outcomes

No OSCC was confirmed in the total screened population of 1111 high-risk individuals but 17 patients were diagnosed with PMDs ranging from OSF to lichenoid changes in addition to lesions with histologically confirmed dysplastic changes. 15 screen positive patients failed to attend for definitive diagnosis despite the best efforts of the cultural advocates and hospital teams.

Benign oral mucosal hyperkeratosis was by far the most common lesion diagnosed in 52.9% of screen positive individuals who attended for definitive diagnosis. In this population most suspected OSCC would be in individuals with a paan chewing habit where the rough fibres of the betel quid may cause frictional damage to the epithelial surface of the mucosa, thereby presenting as hyperkeratotic white patches. Additionally, the presence of lime and other irritants in the paan preparation could cause chemical irritation to the mucosa which would

also result in a hyperkeratotic reaction of the tissue. The keratosis would be histologically classified as benign if there was no evidence of dysplastic change despite the patient's associated risk factors. However, because of these risk factors 73% of the referred patient's remain under review even if initially diagnosed with a benign lesion.

3-4.03 Poor Compliance with Referrals

Unfortunately, just 51 (78%) of the 65 individuals referred to the Department of Oral Medicine attended for further investigation of their suspicious oral mucosal lesions, leaving 15 screen positive individuals without definitive diagnosis (Table 3-05 and Table 3-06). This was despite the best efforts of the advocates to phone and visit these patients as well as offering to attend at their hospital appointment. Although far from ideal this compliance rate is comparable to the published evidence and in particular compares favourably to the 63% referral compliance reported in the Trivandrum Oral Cancer Screening Study [5] . In contrast, screening programmes in the UK usually report very high levels of compliance, approaching 100% [62]. The referral compliance rate was 70% in the 2006 Tower Hamlets screening programme and any improvement in 2008 can be attributed to the extensive and proactive measures taken to ensure compliance and cultural acceptability. The referral compliance rate in UK studies generally being substantially higher than the Trivandrum study may be attributable to the use of qualified dental personnel which anecdotal experience suggests increases patients' confidence in the screening diagnosis and therefore they may be more amenable to further investigation in comparison to the diagnosis of non-dental personnel. The screening dentist's reported patients often taking the opportunity to

ask related dental questions during their screening as many of the patients at risk of OSCC also had other dental disease. The overall percentage of missed outpatient appointments at Bart's and The London is 21.4% [117] which is broadly in line with the findings of this trial and suggests that there are institutional barriers to access in addition to cultural features of the population.

Regrettably, there are still 15 individuals with suspicious oral mucosal lesions who have refused to attend for further investigation, in spite of all reasonable attempts by both the secondary referral centre and bilingual cultural advocates who have experience of dealing with health access issues in the local community. As in 2006, reasons for non-attendance at referral appointments were investigated. It was determined that two of these non-compliers had permanently returned to Bangladesh. Whether this was in any way related to the screen positive OSCC result in unclear as there is known to be a high population turnover in this community.

Overall these results indicate that the model of OSCC screening, in a community setting with ethnically-matched advocates was able to overcome some specific barriers to access for the South Asian population, that remain at the hospital secondary referral service. However, there is significant room for further improvement in compliance with referrals to match that observed in other UK populations. One solution is to have the definitive diagnostic process carried out in the community which would be possible on a mobile dental unit. Our results indicate that on average 3-4 people were screened positive per session and 90% required biopsy to confirm diagnosis. Therefore, a second clinician available to

undertake the biopsy would eliminate the risk of patients not attending the secondary referral centre. The assumptions being that patient's would agree to an immediate biopsy and will attend the hospital for further treatment if required.

3-4.04 Data Collection Effects on Screening

In total 835 people were screened during the 24 sessions held in Tower Hamlets, an average of 35 per session, with the most screenings in a single session occurring on Bethnal Green Road, as in the 2006 programme (Table 3-01 and Table 2-01). The average number of patients seen per session in 2006 was substantially higher at 48 indicating that the data collection process may have reduced the dentist's capacity to screen patients. However, the five Newham sessions yielded an average of 55 people screened per session which taking into account the extra half-hour of screening time at Newham would indicate a Newham screening rate almost equivalent to that in Tower Hamlets 2006 programme. This variance maybe due to the other data being collected in Tower Hamlets by link workers and tobacco cessation workers from Bangladeshi people after screening. This may have interfered with their ability to recruit patients into screening thereby reducing the numbers screened at Tower Hamlets where there were proportionally more Bangladeshi individuals. Conversely the data collection by the dentists didn't affect the screening rate because it was information routinely elicited when assessing cancer risk. Additionally, dentist AL speaks Punjabi so would have been able to communicate directly with many of the Newham locals without an interpreter whilst dentist NS doesn't. This may account for dentist AL screening more patients per session than dentist NS at Newham although dentist NS only attended one session. Approximately equivalent numbers were screened

by both dentists at the sessions in Tower Hamlets and neither dentist speaks Sylheti.

Data collection when integrated into the screening process does not interfere with the running of a screening programme but information collection in addition to that normally elicited in the clinical consultation appears to be detrimental to the primary objective of OSCC screening.

3-4.05 Targeting High Risk Individuals

In Tower Hamlets and Newham all of the screening sites yielded referrals for suspected OSCC. In 2006 three screening sites resulted in no screen positive individuals. This indicates that the site selection policy for Phase II was better at targeting high-risk individuals even if fewer were screened at each session in Tower Hamlets. In addition, there maybe some order-effect as the awareness of the screening programme resulted in the Spitalfields Medical Practice actively recruiting its patients, to be screened. Consequently, the 19% referral rate from this site was substantially higher than the average of 6.6% in Tower Hamlets (Figure 3-05).

Demographic data also suggests the population screened were at high-risk (Table 3-03). The screened population in Tower Hamlets were almost exclusively South Asian (91%) and then predominantly of Bangladeshi origin (84%). The population screened in Newham were more heterogeneous but also majority South Asian (66%), although a more diverse group of Indian's, Pakistani's and Bangladeshi's. The linking of South Asian population's lifestyle risk factors to OSCC risk is

confirmed by 58% of the homogenous Bangladeshi Tower Hamlets group using paan and 44% smoking. Although, just 21% of the Newham cohort were paan users, 37% smoked and 31% drank alcohol. There was no attempt at quantification of these risk factors for the majority of screened individuals unless screened positive but overall the demographic and risk factor descriptive data would suggest the screened population displayed high levels of lifestyle risk factors for OSCC.

Further evidence comes from the dentists screen positive referral rate. In the 2006 Tower Hamlets campaign the overall referral rate was 4.1% whereas in 2008 it was 6.6% so it would appear more high-risk patients are being screened because of the higher referral rate. Dentist AL was involved in both programmes and refereed 2% of screened patients in 2006 compared to 6% in 2008 but this may also be partially due to gaining more experience of OML diagnosis in the interim.

Overall it would appear that OSCC screening by (1) use of a mobile dental unit at community facilities with high footfall of South Asian people and (2) active recruitment of individuals displaying high risk behaviours by bilingual link workers, specifically targets the high-risk South Asian population.

3-4.06 Inter-Examiner Variability

In contrast to the patients attending for screening, the level of inter-examiner variability should be a relatively controllable variable. We succeeded in achieving very high levels of correlation between the examiners by a rigorous standardisation and calibration process and utilising fewer examiners with more

specific interest and experience in oral mucosal disease. The independent measures of DMFT assessment and OML detection used for assessment of variability, yielded correlation co-efficients of 0.97 and 0.88 respectively. In addition, the two dentist's referral rate of screen positive individuals was remarkably consistent at 6-7% in Tower hamlets and 4-5% in Newham. This indicates the calibration and standardisation process was robust.

3-4.07 Gold Standard Outcomes

Despite 1111 individual screenings in a high risk population for oral cancer there were no OSCCs detected. Therefore, and as in 2006, it is impossible to assess the results on the basis of the ideal gold standard for one-off oral cancer screening programmes, which is histologically confirmed OSCC. Without a gold standard outcome it is impossible to determine the commonly calculated measures of sensitivity or specificity to describe the effectiveness of a screening process.

Due to ethical concerns with biopsy of normal tissues and OSCC being rare, various 'soft' gold standards have been suggested as more appropriate for UK screening programmes [109]. The simplest is the number of positively screened individuals. In our study, screen positive referral rates varied greatly dependent on the screening dentist (in 2006 for dentist AR ~15% and dentist AL ~2%) but this variance can be reduced by robust standardisation and calibration (in 2008 for dentist NS ~7% and dentist AL ~6%). However, the majority of these screen positive individuals don't actually have OSCC or even PMDs (Table 3-06 and Table 3-07).

The other commonly reported soft gold standard outcome measure is the proportion of screen positive patients subsequently diagnosed (either clinically or histologically) with PMDs or OSCC. This would be inaccurate with a substantial non-compliance rate, as in the studies on South Asian populations, and relies heavily on the subjective opinion of the specialist clinician charged with making the gold standard diagnosis, if histology is not indicated. This subjectivity maybe little better, in visual diagnosis of OMLs, than the screening clinicians and even if biopsy is indicated there are issues with where and how to accurately biopsy a large lesion. All outcome measures reliant on a definitive diagnosis will have similar issues.

With the known uncertainties in OSCC pathogenesis and diagnosis an alternative gold standard is the requirement for a biopsy in addition to visual assessment to confirm the diagnosis. As visual detection alone is utilised during screening the rationale is that malignancy could not be satisfactorily excluded without the biopsy. In 2008, 90% of referred patients, who attended the secondary referral centre, required a biopsy (Figure 3-11), considerably more than the 57% in 2006 (Figure 2-15) indicating this may be an appropriate measure of a more effective screening campaign.

To negate the need for a definitive diagnosis an appropriate soft gold standard could be, whether or not a screen positive individual is discharged immediately following definitive diagnosis or requires long term follow-up. This is the clinical decision the specialist would need to make based on a number of interplaying factors including the suspected diagnosis and the patient's risk factors. It would be

heavily dependant on the healthcare system but is readily applicable in the UK where healthcare is free at the point of delivery, in secondary referral centres. In 2006, 50% of referred patients were discharged after definitive diagnosis because lesions were benign and there was minimal risk of future malignancy developing (Figure 2-16), whilst in 2008 just 27% were able to be discharged from further follow-up, at 1 year (Figure 3-12). This suggests that patients at higher risk of malignancy were referred in 2008 and in the UK healthcare system there is an obligation to follow-up these patients as well as reducing their acquired risk factors for OSCC.

The sensitivity and specificity of visual screening for oral cancer has repeatedly been reported as sufficient to justify screening programmers despite the lack of robust evidence in the literature [64, 118]. In the UK, in view of the complex presentation of PMDs particularly in South Asian populations, a successful outcome of screening should be considered as a screen positive individual who requires long term follow-up, specifically for development of OSCC, due to the presence of PMDs or significant risk factors. The well reported deficiencies of a 'soft' gold standard are acknowledged as this definition accommodates the clinical judgement of the specialist in oral oncology at the secondary referral centre but is the most appropriate strategy for UK based screening activity.

Table 3-07: 4x4 table of screening outcomes using the gold standard measure of whether individuals require long term follow-up for OSCC. Assuming the worst case scenario that all patients who fail to attend the secondary referral centre would require review.

	review	discharge
screen +tve	52	14
screen -tve	0	1045

On this basis OSCC screening specificity was 98.7% (1045 out of 1059) and positive predictive value 79% (52 out of 66) with 'sensitivity' and 'NPV' at 100% as would be expected from any definition of a gold standard that only assesses the positive screened individuals. For patient safety we assume the worst case scenario that all patients who did not comply with referral would have been reviewed and not discharged. These figures are comparable to the literature on OSCC screening where overall specificity is 97% and PPV 70% despite the disparity in gold standard outcomes utilised and heterogeneity of study methodologies [62]. The 79% PPV indicates that approximately 1 in 5 screen positive individuals were false positives attributable to specific difficulties in assessing the screened population, such as differentiating discrete lesions from generalised paan-related staining. Importantly, high specificity indicates very few false negatives amongst the discharged (healthy) population suggesting few patients who actually require follow-up would be misdiagnosed.

Qualitatively, it may be considered surprising that the screening dentists referred 14 patients with no significant pathology. However, this would be consistent with the varied presentation of OSCC in high-risk individuals and the inability of the examining clinician to review patients prior to referral because of the 'one-off' nature of this screening protocol. It would be normal practice for a clinician, in another dental scenario, to review a PMD before referral e.g. they may reduce a denture base or ask the patient to not wear the prosthesis for a week to eliminate the possibility of constant light trauma as the aetiology for a persistent ulcer. This opportunity was not available in the one-off opportunistic screening methodology

as the patient can either be referred or discharged therefore a persistent ulcer, as per the NICE guidelines, would need to be referred.

3-4.08 Financial Considerations

The cost per screened individual was calculated at approximately £24 including all associated direct costs such as staff and hire of fully equipped mobile dental units but not including consultant time and hospital facilities for follow-up appointments. This cost is approximately half the figure for the NHS breast screening programme which stands at £45.50 per screen, suggesting targeted OSCC screening may be financially viable or at least is not overtly cost-prohibitive. Additionally, there is potentially financial scope to provide additional diagnostic services that may eradicate the uncertainty of direct visual screening for OSCC.

3-5.01 CONCLUSION

The use of mobile dental units located in community settings and supported by cultural advocates offers an effective means of targeting high-risk South Asian populations for OSCC screening. The study methodology offers insights for the further development of oral cancer screening interventions for disadvantaged communities in the UK.

The main concern is that despite implementing all feasible proactive measures such as remaining in contact by phone and visiting patients as well as offering to attend at their hospital appointment a significant proportion of screen positive individuals still fail to attend the secondary referral centre. This results in patients who have been told they need further investigation at an oral cancer screening event, not receiving the healthcare they need. The psychological and social impact of this is unknown but potentially very serious. Secondarily, the resultant incomplete data collection leads to inaccuracies in the evaluation of the screening programme.

To undertake further screening for OSCC in high-risk South Asian populations, mechanisms must be implemented to prevent drop-out of screen positive individuals.

CHAPTER FOUR

THE PREVALENCE OF ORAL MUCOSAL LESIONS IN THE SCREENED POPULATION TARGETED AS HIGH-RISK FOR ORAL CANCER

Chapter 4

The Prevalence of Oral Mucosal Lesions in the Screened Population Targeted as High-Risk for Oral Cancer

4-1.01 INTRODUCTION

It was observed in the 2006 pilot screening programme that many screened individuals presented with OMLs particularly in relation to OSCC risk habits such as paan usage and smoking, which made visual examination more difficult for the dentist. However, as minimal data was collected in the pilot project, to preserve anonymity, it was not possible to evaluate this observation. In the 2008 Phase II screening, more data collection was undertaken in order to determine if those screened were actually at high-risk for OSCC. Demographic data discussed in Chapter 3 suggests that the screened population was at high-risk due to the reported levels of OSCC risk behaviours such as paan usage and smoking. However, an assessment of the prevalence of oral mucosal lesions in the screened population would be necessary to confirm this as most OSCCs develop in PMDs presenting as visible lesions of the mucosa [29]. Additionally, early OSCC, being a cancer of the superficial epithelial lining of the intraoral mucosa should be detectable as a mucosal abnormality even if not presenting as a typical PMD.

4-1.02 Direct Visual Examination and Oral Cancer

The literature records that oral cancer can be detected by direct visual examination of the oral mucosa with overall sensitivity 85%, specificity 97%, PPV 70% and NPV 98% [62]. The low PPV indicates direct visual examination is likely to

result in false positives which are attributed to the heterogeneity of early OSCC and particularly PMD clinical presentations. The number of false positives will further be potentiated by the rarity of OSCC in the population. The presence of large numbers of OMLs or specific PMDs adversely affects the screening clinician's ability to accurately detect OSCC on direct visual examination, potentially indicating this modality is inappropriate for such populations without diagnostic aids.

4-1.03 Leukoplakia

Much of the literature on PMDs focuses on leukoplakia as one of the commonest recognised potentially malignant OMLs in the Western World. The term leukoplakia is defined as "white plaques of questionable risk having excluded (other) known diseases or disorders that carry no increased risk for cancer" [15]. Leukoplakia is a clinical term with no specific histological presentation and additional clinical descriptions that are used to assist in the characterisation of leukoplakia are:

- Aetiological description e.g. clearly associated with tobacco or areca nut use or idiopathic.
- Site descriptions e.g. giving anatomical sub-site in the mouth or oropharynx
- Size or extent of the lesion(s).

Clinical diagnosis of leukoplakia is outlined in the schematic presented in Figure 4-01 from Warnakulasuriya et. al. (2007) reporting on a WHO Collaborating Centre for Oral Cancer and Precancer workshop [15].

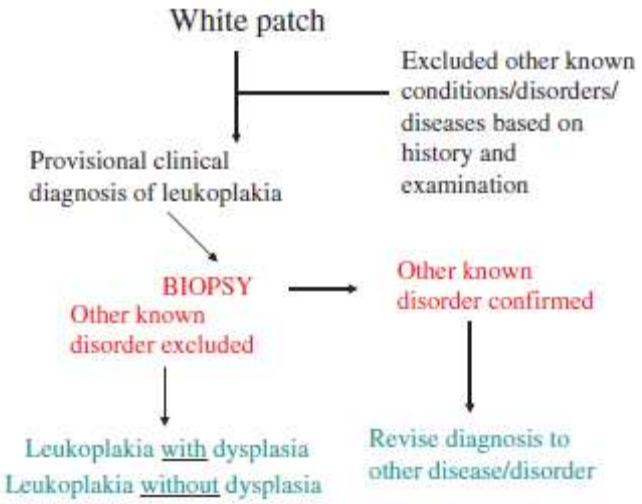


Figure 4-01: Schematic representation of the steps in diagnosis of oral leukoplakia. (from Warnakulasuriya et. al 2007 [15])

The WHO Working Group also provided an extensive list of other possible diagnosis which need to be excluded for white patches to be classified as leukoplakia (Table 4-01).

Table 4-01: Disorders that need exclusion to diagnose leukoplakia. (from Warnakulasuriya et. al 2007 [15])

Disorder	*Diagnostic features*	*Biopsy*
White sponge nevus	Noted in early life, family history, large areas involved, genital mucosa may be affected	Biopsy not indicated
Frictional keratosis	History of trauma, mostly along the occlusal plane, an etiological cause apparent, mostly reversible on removing the cause	Biopsy if persistent after elimination of cause particularly in a tobacco user
Morsicatio buccarum	Habitual cheek – lip biting known, irregular whitish flakes with jagged out line	Biopsy not indicated
Chemical injury	Known history, site of lesion corresponds to chemical injury, painful, resolves rapidly	Not indicated
Acute pseudomembranous candidosis	The membrane can be scraped off leaving an erythematous/raw surface	Swab for culture
Leukoedema	Bilateral on buccal mucosa, could be made to disappear on stretching (retracting), racial	Not indicated
Lichen planus (plaque type)	Other forms of lichen planus (reticular) found in association	Biopsy consistent with lichen planus
Lichenoid reaction	Drug history, e.g. close to an amalgam restoration	Biopsy consistent with lichen planus or lichenoid reaction
Discoid lupus erythematosus	Circumscribed lesion with central erythema, white lines radiating	Biopsy consistent with DLE supported by immunofloresence and other investigations
Skin graft	Known history	Not indicated
Hairy leukoplakia	Bilateral tongue keratosis	Specific histopathology with koilocytosis; EBV demonstrable on ISH
Leukokeratosis nicotina palate	Smoking history, greyish white palate	Not indicated

4-1.04 The Prevalence of PMDs

Petti (2003) [119] analysed the literature on prevalence of PMDs, focussing on leukoplakia and the much rarer erythroplakia, and calculated a global prevalence of between 1.49% (95% CI, 1.42-1.56] and 2.6% (95% CI: 1.72–2.74) dependent on methodology. There is no equivalent figure in the literature for other PMDs and only assessing leukoplakia and erythroplakia is likely to significantly underestimate the prevalence of PMDs in the Tower Hamlets and Newham populations as other risk factor specific conditions, such as OSF, are significant issues in these South Asian populations.

4-1.05 PMDs in the UK Bangladeshi Population

Pearson et. al. (2001) [23] conducted a screening activity amongst Bangladeshi general medical practice patients in Tower Hamlets. Over a period of 3 months, 137 patients aged over 40 were approached in the medical practice waiting room and agreed to take part in a structured interview before undergoing an oral visual examination. They detected OMLs in 40% of participants with no gender predilection (Table 4-02). Of all detected lesions, 64% (n = 34) were leukoplakia and these presented in 25% of the screened population, with the next most common lesion being acute abscess (n = 6) followed by the related dental pathology of a sinus (n = 4). OSF was detected in 1 female patient. Ulcers were reported in 3 females but not specifically as suspicious for OSCC, although a separate category for ulceration due to dentures was recorded. Benign mucosal conditions, such as aphthous stomatitis are much more likely to present as oral ulceration than malignancy, especially as aphthous stomatitis is commoner in women. Therefore, from their list of identified OMLs those that can be classified as

PMDs make up 67-73% (n = 37-40) of all OMLs, depending on whether the 3 ulcers were included as suspicious.

Table 4-02: Oral mucosal lesions detected in a screened Bangladeshi population in Tower Hamlets. (from Pearson et. al. (2001) [23])

Oral lesions	Male (Base=77)		Females (Base=60)		Total (Base=137)	
	n	%	n	%	n	%
Acute abscess	1	1	5	8	6	4
Angular cheilitis	–	–	1	2	1	1
Denture stomatitis	2	3	–	–	2	1
Erythroplakia	1	1	–	–	1	1
Frictional keratosis	3	4	–	–	3	2
Leukoedema	1	1	–	–	1	1
Leukoplakia	18	23	16	27	34	25
Lichen planus	–	–	1	2	1	1
Nicotinic stomatitis	2	3	–	–	2	1
Sinus	3	4	1	2	4	3
Submucous fibrosis	–	–	1	1	1	1
Ulcer	–	–	3	5	3	2
Ulceration due to dentures	2	3	–	–	2	1
Total	31	40	24	40	55	40

NB: The total number of participants with oral mucosal lesions is less than the sum of specific lesions because some participants had more than one lesion

Even amongst medical practice users Pearson et. al. (2001) [23] reported paan usage rates at 88% amongst females and 69% in males whilst 60% of females and 71% of males used tobacco, either smoked or chewed. Alcohol consumption was not reported but with the over 40 age criteria and the reported risk factors this would suggest the majority of the screened population were high-risk for OSCC.

This study has significant methodological differences to our targeted screening protocol that may make comparison of results difficult. Primarily, they did not actively recruit high-risk individuals but simply inviting those in a general medical practice waiting room to take part. Their screened population maybe health conscious because they are recruiting from medical practice attenders or

conversely the sampled population maybe chronically ill and therefore not representative of our targeted high-risk screened population.

Despite the variety of studies on the prevalence of risk factors for OSCC in the Tower Hamlets, Newham or East London populations, any other studies of OML prevalence were scarce in the literature. An abstract by Haque et. al. 1997 [120] reported an OSF prevalence of 0.7% amongst 150 individuals in the Bangladeshi population of East London but methodology was unclear in the limited write-up. Even broadening the search criteria to Asian populations in the UK revealed just one further study reported by Mattin (1989) [121] in a University of London MSc Thesis. In a predominantly Indian origin population based in Southampton they recorded OMLs in 19% of patients, with frictional keratosis related to denture wear, the commonest finding. Two subjects were reported with paan staining of the oral mucosa and OSF prevalence in the sample was 0.7%.

4-1.06 PMD Prevalence in South Asia

Studies from various regions of India predominate in the literature search for prevalence of OMLs in South Asian populations outside the UK. There were no reports from Bangladesh. The Trivandrum Oral Cancer Screening Study [2-6] does not report the prevalence of OMLs but risk factors for OSCC are recorded. Chewing habits are reported in approximately 25% of males and females whilst smoking is prevalent in 60% of males and just 1% of females. Drinking habits were reported in 40% of males and 0.1% of females. This information was collected in the patient's home and may reflect cultural acceptability of the different habits amongst men and women.

Saraswathi et. al. (2006) [24] reported on 2017 individuals seen in a Chennai hospital dental department in a 3 month period. OMLs were detected in 4.1% with leukoplakia in 0.59% and OSF in 0.55% (Table 4-03). The commonest lesion was the benign hyperpigmentation smoker's melanosis in 1.14% and stomatitis nicotina in 0.89% although the prevalence of smoking was 15.02%. A chewing habit was prevalent in 8.78% and alcohol use by 6.99%.

Table 4-03: Oral mucosal lesions detected in a screened population in Chennai, India. (from Saraswathi et. al. (2006) [24])

Prevalence of soft tissue lesions by gender

Lesions	Prevalence in Men (%)	Prevalence in Women (%)	General Prevalence (%)
Smoker's Melanosis	1.63	0.27	1.14
Leukoplakia	0.7	0.41	0.59
Stomatitis Nicotina Palatini	1.24	0.27	0.89
Leukedema	0.39	0	0.25
Chewer's Mucositis	0.23	0.27	0.25
Oral Submucous Fibrosis	0.62	0.41	0.55
Median Rhomboid Glossitis	0.38	0	0.25
Lichen Planus	0.23	0	0.15
Candidiasis	0.07	0	0.05
Number of study Participants	**1287**	**730**	**2017**

Byakodi et. al. (2011) [25] reported on 24422 individuals seen at a hospital dental outpatient department in Sangli, India, in an 18 month period. OMLs were detected in 2.55% with aphthous ulcers the commonest lesion in 0.80% followed by OSF in 0.68%, leukoplakia in 0.36% and OSCC in 0.34% (Table 4-04). The reported risk habits were 9% of the population smoked, 23 % chewed tobacco and 18% were described as 'alcoholics'.

Table 4-04: Oral mucosal lesions detected in a screened population in Sangli, India. (from Byakodi et. al. (2011) [25])

The prevalence of oral lesions in the study population

Lesions	Male	Female	Total
Apthous ulcer	75	120	195
Leukoplakia	56	19	75
Oral submucous fibrosis	146	02	152
Leukoplakia + oral submucous fibrosis	12	0	14
Oral lichen planus	21	09	35
Oral cancer	61	21	82
Denture stomatitis	10	13	23
Fibroma	14	10	28
Pyogenic granuloma	05	14	19
	415 (66.61%)	208 (33.39%)	623

Mehrotra et. al. (2008) [122] reported on 3030 people screened on 10 days at a hospital dental department in Vidisha, India. OMLs were detected in 8.4% but 16% refused scalpel biopsy and the clinical diagnosis were not presented. Risk factors were, 21% smoked and 42% were tobacco chewers with alcohol usage not reported.

Other studies have targeted specific South Asian population groups, usually believed to be a higher risk of OMLs, and not just the attendees at a hospital dental department as in the three studies above. In Rajasthan state, Dagli et. al. 2008 [123] reported leukoplakia in 33.3% of 513 mine workers and OSF in 1.8% with 59% using tobacco and 60% using alcohol. This study did not report on any other specific OMLs except papilloma, which was present in 1.8%. Although the presentation is unclear there is some data suggesting that 324 of the 513 people

screened presented with some OMLs indicating that the total prevalence of OMLs was 63%.

Despite the heterogeneity evident in these studies when taken together the prevalence of OMLs in Indian populations presenting to hospital outpatient departments is likely to be in the range 2 - 8% with common PMDs including OSF (0.5 - 0.7%) and leukoplakia (0.3 - 0.6%). The reporting of other non-PMD but common OMLs appears inconsistent as smoking related changes are common in the Chennai study but not reported at all in Sangli despite 9% of the screened population smoking. Conversely aphthous ulceration is the commonest lesion in Sangli but is not recorded at all in Chennai. Chewing habits are relatively common in both populations but chewer's mucositis is reported in 0.25% of the Chennai population and not at all in Sangli. These studies have large enough populations to believe that common OMLs should occur in both without methodological weaknesses, unless the studied populations are significantly different. Additionally, when assessing specific high-risk Indian populations, such as mine-workers, there is some evidence that the prevalence of OMLs maybe much higher.

4-1.07 Summary

Assessment of the prevalence of abnormalities of the oral mucosa present in the screened population is necessary to determine if the screened population is truly at high-risk of OSCC. The literature gives no reliable indication of the prevalence of OMLs in the Tower Hamlets and Newham screened populations as no studies have attempted to specifically target individuals with OMLs. This suggests the available literature may significantly underestimate the prevalence in our screened

population. Importantly for future screening programmes, the presence of large numbers or a substantial range of OMLs is likely to be the cause of misdiagnosis and resultant reduced accuracy of OSCC screening by direct visual examination in this population.

4-2.01 STUDY PROTOCOL

A population-based survey was designed to record the prevalence of OMLs in those screened for OSCC without adversely interfering with the primary objective. The pilot study revealed that at busy screening locations, potential patients were being kept waiting and tended to leave the mobile dental unit without being screened, if they were not seen within a few minutes. Each screening was to last no more than 3-4 minutes on average and therefore data collection had to be optimised to avoid extending the screening time.

Data collection was approved by The East London and the City Research Ethics Committee and the Joint Research and Development Office of Bart's and The London (REC 08/H0701/98). Data analysis utilised Excel 2003 (Microsoft, USA) with the StatPro add-on (Kelley School of Business, Indiana University) and STATA 10 (StataCorp LP, Texas, USA). To allow for anonymity all participants initially gave verbal and implied consent to take part in screening but written consent was obtained for recording of patient related data for all screened patients, if they agreed for data to be collected. Refusing to participate in the data collection exercise did not prevent patients from being screened or referred. No attempt was made to elicit why participants may not wish to allow recording of their data.

4-2.02 Clinical Diagnosis

The clinical diagnosis or a description of mucosal pathology, if diagnosis was unclear, of all OMLs was to be recorded whether referred or not. Standardisation was aided by developing a defined list of OML clinical diagnosis based on the

recently up-dated nomenclature by Warnakulasuriya et. al. (2007) [15] and the list utilised by Pearson et. al. (2001) [23] in their study of the Tower Hamlets Bangladeshi population (Table 4-02). Other lesions and conditions were as described in Cawson's Essentials of Oral Pathology and Oral Medicine 7th Edition (2002) [8]. The list was not exhaustive and was to be updated by agreement between both examiners, as necessary throughout the screening programme. The final list of OML clinical diagnosis is shown in Table 4-05.

Table 4-05: Agreed clinical diagnosis of oral mucosal pathology to standardise description of lesions detected in the screened population.

WHITE PATCHES	ULCERS
CANDIDOSIS +/- ACUTE PSEUDOMEMBRANOUS etc	. APHTHOUS ULCER +/- MINOR / HERPETIFORM / MAJOR
ERYHTROLEUKOPLAKIA	ULCER +/- OSCC / TRAUMATIC / CHEMICAL etc
FRICTIONAL KERATOSIS	
LEUKOEDEMA	**LUMPS**
LEUKOPLAKIA	DENTAL ABSCESS
LICHENOID REACTION	EPULIS
LICHEN PLANUS (LP) +/-. RETICULAR / PLAQUE etc	FIBROEPITHELIAL POLYP (FEP) / PYOGENIC GRANULOMA
SINUS TRACT	MUCOCELE
WHITE SPONGE NAEVUS	OSTEOMA (BUT NOT LINGUAL/PALATAL TORI)
RED PATCHES	**SPECIFIC PALATAL LESIONS**
ERYHTROPLAKIA	DENTURE STOMATITIS
HAEMANGIOMA	SMOKERS PALATE
OTHER COMMON CONDITIONS	**SPECIFIC TONGUE LESIONS**
ANGULAR CHEILITIS	GEOGRAPHIC TONGUE
DESQUAMATIVE GINGIVITIS	ATROPHIC GLOSSITIS
GINGIVAL HYPERPLASIA	MEDIAN RHOMBOID GLOSSITIS
ORAL SUBMUCOUS FIBROSIS (OSF)	
PAAN STAINING	

Where multiple mucosal abnormalities are related to one pathological process the examiners agreed to record all detectable lesions e.g. in OSF there would usually be paan staining and may also be dental disease presenting as a mucosal sinus

tract. This would allow an accurate measure of the prevalence of all mucosal abnormalities as well as specific lesions in the screened population and give an idea of difficulty in assessing this population for OSCC. Paan staining specifically was recorded as it was reported to complicate OSCC detection and clinical diagnosis by the dentists in the 2006 Tower Hamlets pilot project. It can also be difficult to differentiate paan staining from inflammatory paan-related mucositis without histological evaluation.

4-2.03 Intraoral Site

As OSCC predominantly develops at specific sites in the mouth it is believed that OMLs at different sites carry different risks of malignant conversion [29]. Therefore, the site of lesions was recorded on a mouth map to permit assessment of the proportion of OMLs at high risk sites (Figure 4-02).

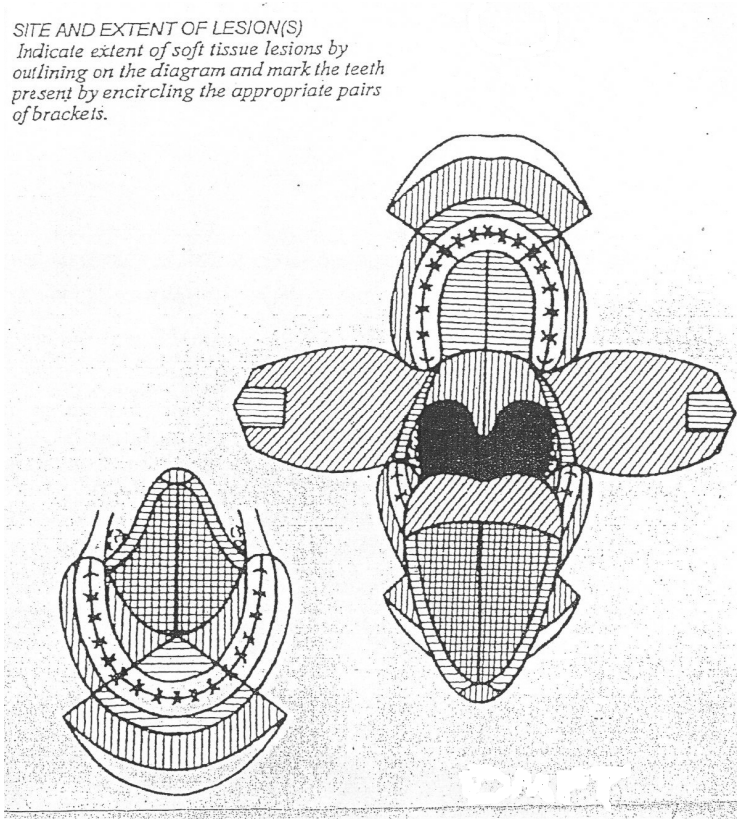


Figure 4-02: Mouth map used to delineate position and extent of oral mucosal lesions detected in the screened population.

Where discrete lesions were detected they would be noted at each site e.g. a 1cm x 1cm leukoplakia on the right buccal mucosa and another 0.5cm x 1cm on left floor of mouth in the same patient would be recorded as two leukoplakias whilst conditions would be recorded per patient e.g. reticular lichen planus classically presenting as bilateral buccal mucosal lesions would be recorded as 1 condition, unless there was, for example, an associated desquamative gingivitis, in which case they would be recorded as two conditions at two different sites (i.e. buccal LP and gingival desquamative gingivitis).

Extra-oral examination findings were not recorded unless directly relevant e.g. lymph node involvement suggesting metastatic spread of an OSCC, in order to avoid the screening consultation taking too long.

4-2.04 Risk Factors for Oral Cancer

All patient screened for OSCC were asked about their risk habits, including smoking or areca nut extract usage or alcohol consumption as this is part of the risk assessment process for OSCC. Recording this marginally increased screening session time because the information would usually only be obtained verbally unless referral was indicated. No specific attempt was to be made to determine the constituents of paan i.e. whether tobacco was included, unless referring a screen positive individual where the risk factors would be discussed in greater detail in the normal circumstance. Similarly with smoking and alcohol consumption, quantification would not be attempted (pack years, ounces of tobacco, use of filters, units of alcohol etc) for every patient.

4-3.01 RESULTS

The patient demographic data and risk factor prevalence of the 1111 screened participants in Tower Hamlets and Newham is presented and discussed in Chapter 3.

4-3.02 Numbers of Detected Oral Mucosal Lesions

In total 1438 OMLs were detected in 604 (54%) individuals in the screened population of 1111 individuals (Table 4-06).

Table 4-06: All mucosal lesions detected in the screened population.

WHITE PATCHES	LEUKOPLAKIA	60
	ERYHTROLEUKOPLAKIA	11
	LICHEN PLANUS (RETICULAR)	41
	LICHEN PLANUS (EROSIVE)	1
	LICHENOID REACTION	6
	CANDIDOSIS (ACUTE PSEUDOMEMBRANOUS)	10
	FRICTIONAL KERATOSIS	520
	LEUKODEMA	74
	WHITE SPONGE NAEVUS	1
	FORDYCE SPOTS	29
	SINUS TRACT	24
RED PATCHES	ERYHTROPLAKIA	5
	HAEMANGIOMA	2
ULCERS	ULCER (OSCC)	3
	ULCER (TRAUMATIC)	15
	MINOR APHTHOUS ULCERS	25
	HERPETIFORM APHTHOUS ULCERS	1
LUMPS	MUCOCELE	2
	FIBROEPITHELIAL POLYP (FEP) / PYOGENIC GRANULOMA	11
	EPULIS	1
	DENTAL ABSCESS	4
	OSTEOMA (NOT LINGUAL/PALATAL TORI)	2
SPECIFIC PALATAL LESIONS	DENTURE STOMATITIS	10
	SMOKERS PALATE	39
SPECIFIC TONGUE LESIONS	GEOGRAPHIC TONGUE	3
	MEDIAN RHOMBOID GLOSSITIS	2
	ATROPHIC GLOSSITIS	1
OTHER SPECIFIC CONDITIONS	PAAN STAINING	522
	DESQUAMATIVE GINGIVITIS	1
	ANGULAR CHEILITIS	2
	ORAL SUBMUCOUS FIBROSIS (OSF)	7
	GINGIVAL HYPERPLASIA	3
TOTAL NUMBER OF LESIONS DETECTED		1438

A total of 32 different OMLs were diagnosed in the population ranging from unquestionably benign leukoedema to the possibly already malignant erythroplakia.

4-3.03 Common Lesions in the Screened Population

Frictional keratosis and paan staining were by far the commonest lesions detected, comprising 522 (36%) and 520 (36%) of all OMLs respectively. Leukoedema was the next most common OML detected in 74 people consequently comprising 5.1% of all OMLs (Table 4-06). Leukoedema is an entirely benign normal variation of buccal mucosa but is an important finding as it has been reported to appear as a false positive result when examined with light based tissue reflectance aids to oral visual assessment [81].

Only 23 cases (1.6% of all OMLs) of the apparently common condition minor aphthous ulceration were detected. This could be because of the sporadic nature of these ulcers but the condition also predominantly affects younger people so this low prevalence might be expected when targeting older individuals. Minor aphthous ulcers are also known to be uncommon in smokers [8] so the high rates of smoking reported may provide another explanation for the infrequency of detection.

4-3.04 Prevalence of Leukoplakia

Sixty leukoplakias were reported, representing 4.1% of all OMLs detected in the screened population (Table 4-06). This is considerably lower than that reported by the 2001 Pearson et. al. (2001) [23] study in Tower Hamlets and more consistent

with the later trials reported in 2006 and 2011 in India [24, 25]. This may in part be due to the updating of the definition of leukoplakia since 2001 [15] so some clinically detectable white patches previously called leukoplakia may have been further classified by cause, for example, as frictional keratosis. Some evidence of this comes from the fact that 32 different lesions were reported in our study whilst Pearson et. al. (2001) [23] reported just thirteen different OMLs (Figure 4-02).

4-3.05 Prevalence of Oral Submucous Fibrosis

Seven cases of OSF were detected amongst the 522 people with apparent paan staining (Table 4-06) and 586 people in total reported paan usage (Table 3-03). As quantity and frequency of paan use were not collected the apparent lack of paan staining in 44 individuals who reported paan usage could represent the most infrequent users or those with meticulous oral hygiene. The detection of 7 OSF cases represent 0.5% of all OMLs and 0.6% of all screened individuals which is consistent with the literature on the Bangladeshi population in East London indicating prevalence of 0.7% [120] and 1.8% [23] considering the small sample sizes in these previous studies and the detection of just 1 OSF case in each study.

4-3.06 Detection of Rare Lesions

Some very rare lesions were also detected such as 1 patient with white sponge naevus and another with herpetiform aphthous ulceration (Table 4-06). This could indicate the oral cancer awareness programme, surrounding the screening initiative, was making people look in their mouth for OSCC. There was some qualitative reporting by the clinicians to this effect, as people attended screening sessions specifically for an intraoral lesion they had noticed such as one of the

geographic tongue lesions detected. Fordyce spots are uncommon benign lesions usually presenting in older people, so detection of 29 patients with these lesions would also suggest a proportion of older people were screened.

4-3.07 Referred Patients

The clinical diagnosis at time of screening of the 66 patients referred with suspected cancer is presented, along with the definitive diagnosis (where available) in Table 4-07 for Newham and 4-08 for Tower Hamlets.

*Table 4-07: Definitive diagnosis and screened diagnosis for the 11 patients referred from Newham screening sessions. Shaded screening diagnosis are considered accurate with the definitive diagnosis. (DNA= did not attend secondary referral centre; *****= definitive diagnosis confirmed by histology; #= did not return for biopsy after initial diagnosis at consultant clinic, R= right side, L= left side).*

Patient	Primary Diagnosis	Outcome	Screened Diagnosis
AL210307	hyperkeratosis*	discharge	leukoplakia R FOM + paan staining
AL220304	**oral submucous fibrosis***	review	OSF + leukoplakia R FOM + paan staining
AL220308	DNA	-	leukoplakia R ventral tongue
AL220317	hyperkeratosis*	review	leukoplakia R buccal + paan staining
AL220331	hyperkeratosis*	review	leukoplakia R FOM
AL220363	hyperkeratosis*	discharge	erythroleukoplakia L ventral tongue
NS300302	**oral submucous fibrosis***	review	OSF + leukoplakia R buccal + paan staining
NS300333	candidiasis	review#	leukoplakia L ventral tongue
AL260712	**mild/moderate dysplasia***	review	leukoplakia R FOM + paan staining
AL270754	**lichenoid***	review	leukoplakia R buccal sulcus + LP
AL270771	hyperkeratosis*	discharge	leukoplakia R buccal + paan staining

*Table 4-08: Definitive diagnosis and screened diagnosis for the 55 patients referred from Tower Hamlets screening sessions. Shaded screening diagnosis are considered accurate with the definitive diagnosis. (DNA= did not attend secondary referral centre; *****= definitive diagnosis confirmed by histology; #= did not return for biopsy after initial diagnosis at consultant clinic, R= right side, L= left side).*

Patient	Primary Diagnosis	Outcome	Screened Diagnosis
AL260314	hyperkeratosis*	review	leukoplakia R+L buccal sulcus + paan staining
AL260337	DNA	-	erosive LP L buccal
AL260339	**oral submucous fibrosis***	review	OSF and non-healing ulcer R ventral tongue
AL260342	hyperkeratosis*	review	leukoplakia R buccal sulcus + paan staining
NS310304	DNA	-	leukoplakia R ventral tongue + paan staining
NS310334	DNA	-	leukoplakia R buccal sulcus + R FOM
NS310331	**mild/moderate dysplasia***	review	erythroleukoplakia R FOM + paan staining
NS310330	**lichen planus***	review	erythroleukoplakia R ventral tongue + LP
NS310339	hyperkeratosis*	review	leukoplakia R+L buccal + paan staining
AL020409	DNA	-	leukoplakia R buccal sulcus + paan staining
AL020411	hyperkeratosis	review#	leukoplakia L buccal sulcus + L ventral tongue
AL020430	DNA	-	ulcer (OSCC) R FOM
AL020439	hyperkeratosis*	discharge	erythroleukoplakia L FOM + paan staining
AL020441	**oral submucous fibrosis***	review	OSF + erthyroleukoplakia R FOM/ventral tongue + paan staining
AL020443	haemangioma	discharge	leukoplakia L buccal sulcus + erythroplakia L palate + paan staining
AL050408	DNA	-	leukoplakia R FOM + paan staining
AL050423	hyperkeratosis*	review	leukoplakia R buccal sulcus
AL050431	hyperkeratosis*	discharge	leukoplakia R+L buccal sulcus + paan staining
NS070401	fibro-epithelial polyp*	discharge	erythroleukoplakia R buccal + paan staining
NS070405	DNA	-	leukoplakia R ventral tongue + paan staining
NS070415	hyperkeratosis*	review	leukoplakia R buccal sulcus + paan staining
NS070417	DNA	-	leukoplakia R+L buccal sulcus + gingivae + paan staining
NS070422	hyperkeratosis*	review	leukoplakia R FOM + paan staining
NS070422	**lichenoid***	review	lichenoid + leukoplakia R FOM
NS070423	**lichenoid***	review	leukoplakia R ventral tongue + LP
NS070434	hyperkeratosis*	review	leukoplakia R buccal + paan staining
AL090411	**mild/moderate dysplasia***	review	ulcer (OSCC) R ventral tongue + paan staining
AL090419	hyperkeratosis*	discharge	leukoplakia L FOM + paan staining
AL090421	candidiasis*	review	erythroleukoplakia R buccal + paan staining
NS140418	**mild/moderate dysplasia***	review	erythroplakia L FOM
NS140429	DNA	-	leukoplakia R ventral tongue + paan staining
NS140434	DNA	-	leukoplakia R FOM
AL160402	**mild/moderate dysplasia***	review	erythroplakia L ventral tongue + paan staining
AL160427	**moderate/severe dysplasia***	review	erythroplakia R FOM + paan staining
AL230418	physiological pigmentation	discharge	leukoplakia R buccal sulcus
AL230426	DNA	-	erythroplakia R ventral tongue + paan staining
NS280407	hyperkeratosis*	review	leukoplakia R ventral tongue + paan staining
NS280412	hyperkeratosis*	review	leukoplakia L FOM + paan staining
NS280422	hyperkeratosis*	review	erythroleukoplakia L FOM + paan staining
NS280436	haemangioma	discharge	leukoplakia R buccal + L FOM + paan staining
AL250403	hyperkeratosis	review	leukoplakia R+L FOM + paan staining
AL300401	hyperkeratosis*	review	leukoplakia L ventral tongue + paan staining
AL300413	hyperkeratosis*	discharge	leukoplakia R+L buccal
AL030505	DNA	-	leukoplakia R FOM + paan staining
NS050503	hyperkeratosis*	review	leukoplakia L FOM + paan staining
AL070519	**oral submucous fibrosis***	review	OSF + leukoplakia L FOM + paan staining
AL140528	hyperkeratosis*	review	leukoplakia L FOM + paan staining
AL140539	hyperkeratosis*	review	leukoplakia R+L buccal + paan staining
AL170545	DNA	-	leukoplakia R ventral tongue + paan staining
AL210504	physiological pigmentation	discharge	erythroleukoplakia R ventral tongue + paan staining
AL210516	hyperkeratosis*	discharge	leukoplakia R ventral tongue/FOM
AL230522	**oral submucous fibrosis***	review	OSF + leukoplakia R buccal
NS260516	DNA	-	leukoplakia R FOM
AL280512	**mild/moderate dysplasia***	review	erythroleukoplakia R ventral tongue + paan staining
AL280531	hyperkeratosis*	discharge	ulcer (OSCC) L ventral tongue + paan staining

As no OSCC was detected in those who attended for definitive diagnosis the diagnosis are effectively all false positives. However, as discussed in Chapter 3 the gold standard outcome of ‘patients who cannot be discharged from further follow-up’ is a more appropriate target for screening activity in the UK. The discharged patients have a range of screening clinical diagnosis including leukoplakia, erythroleukoplakia and even erythroplakia confirming the difficulty a screening dentist has in making a clinical diagnosis with PMDs.

51 of 66 patients attended for definitive diagnosis of their screen positive status and Tables 4-07 and 4-08 show 17 of 51 (33%) of screening diagnosis could be considered accurate. The screening diagnosis were most accurate for the more clinically distinct PMDs such as OSF where all 6 cases were accurately reported and LP or lichenoid reactions where all 4 patients were also accurately reported.

4-3.08 Complex and Benign Lesions

Of 1438 oral mucosal lesions detected in the screened population, 54% were clinically diagnosed as benign and no patients with these lesions were referred. This implies the clinicians can readily exclude malignancy even if risk factors are prevalent. Of the 46% of lesions that were not definitively benign only a proportion were referred (Figure 4-03) as either PMDs in high risk individuals or suspected malignancy. The large number of clinically ‘not-benign but also not definitively malignancy’ OMLs detected indicates the difficulty in assessing this high-risk population for OSCC by direct visual examination. These should be termed ‘complex lesions’ to distinguish them from the ‘benign lesions’ and also from the referred PMDs or malignancy.

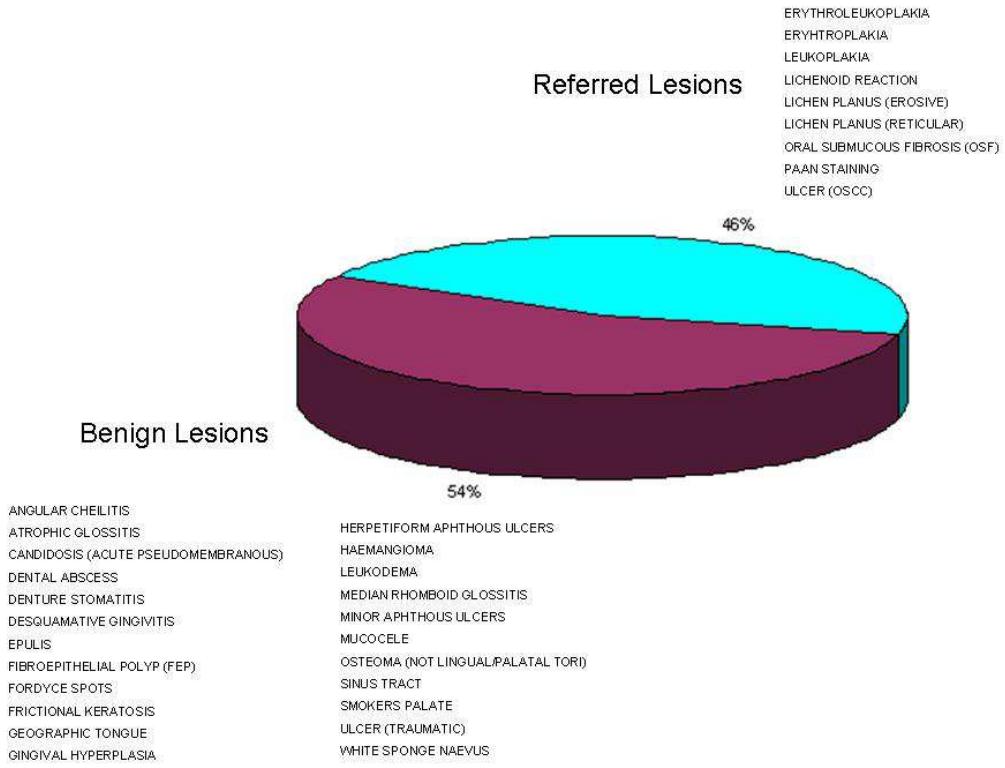


Figure 4-03: The clinically benign lesions detected and the complex lesions, of which some were referred because malignancy could not be excluded by direct visual examination in high risk individuals.

4-3.09 Proportions of OMLs Detected and Referred

Amongst the clinically complex lesions, Figure 4-04 shows that 100% of erythroleukoplakias, erythroplakias and ulcers suspected to be OSCC were referred to the tertiary referral centre. One erosive LP lesion detected was also referred as this would be consistent with the presentation of OSCC. 93% of detected leukoplakias were referred due to the presence of significant risk factors such as tobacco and paan usage with the remainder detected in low risk individuals and therefore classified as routine referrals and inappropriate for a suspected cancer pathway. 67% of lichenoid reactions and 12% of reticular LP were also referred but always when present in conjunction with other lesions more

indicative of OSCC, such as erythroleukoplakia. Six out of 7 patients with OSF were referred because of the presence of associated lesions of concern and the other was also already under the care of the hospital. Paan staining itself was not an indication for referral, as it is endemic in areca nut extract users, but 11% of individuals also had other referable lesions.

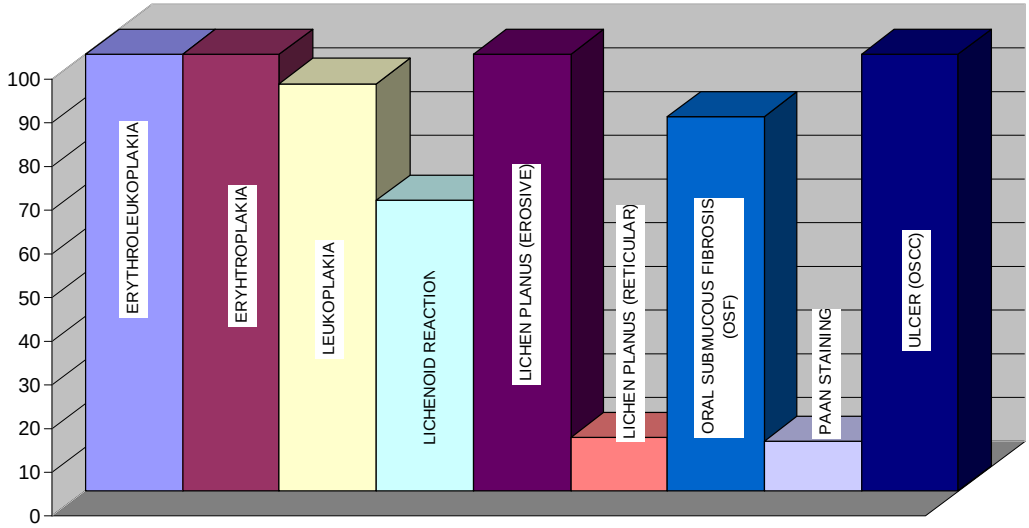


Figure 4-04: The percentage of detected oral mucosal lesions referred to the secondary referral centre.

4-3.10 Site of Lesions

Mucosal lesions were detected throughout the oral cavity (Table 4-09) with the largest proportion (48%) on the buccal mucosa including the buccal sulcus. The buccal area was also the largest single site for the complex (36%) and referred (35%) OMLs. The buccal mucosa is also often the site of frictional keratosis related to the occlusion and therefore was also the single commonest site for benign mucosal lesions. There were no clinically benign lesions reported on the FOM or ventral tongue, which are conventionally high-risk sites for OSCC (Figure

4-05) and there were no referred lesions and very few complex lesions on the dorsum of the tongue consistent with this being a very rare site for OSCC.

Table 4-09: Intraoral site of detected oral mucosal lesions in the screened population presented as the percentage of lesions at each site.

	Percentage of lesions at each intraoral site									
	Buccal mucosa	Labial mucosa	Gingivae	Residual alveolar ridge	Palate	Floor of mouth	Ventral tongue	Dorsal tongue	Lateral/ tip of tongue	Commisure
Referred lesions	35%	6%	7%	0%	1%	30%	21%	0%	0%	0%
Complex lesions	36%	12%	25%	3%	1%	8%	8%	1%	5%	1%
Benign lesions	71%	8%	5%	2%	6%	0%	0%	1%	6%	0%
All lesions	48%	11%	19%	3%	3%	6%	5%	1%	5%	1%

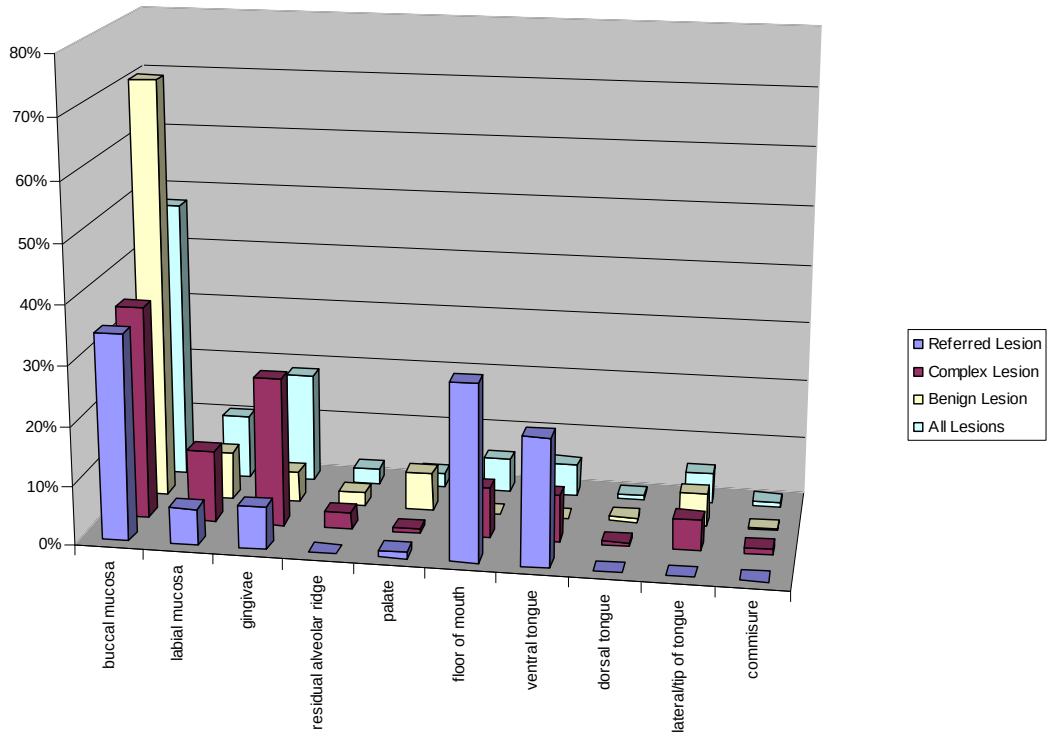


Figure 4-05: Intraoral site of detected oral mucosal lesions in the screened population presented as the percentage of clinically benign lesions, clinically complex lesions and its subgroup of refereed lesions, at each site, as well as all detected lesions shown by site.

4-3.11 Lesion Detection and Gender

The total screened population of 1111 people was 58% male and 42% female however the 1438 lesions detected were predominantly in females (64%) (Table 4-10). Analysing the breakdown of lesions in the referred group when classified as complex or benign, confirmed this female predominance in the detected OMLs. Pearson et. al. (2001) [23] reported a similar male:female mix in the screened population (56:44) but their 55 detected OMLs were reported in the same proportions (56:44) as the population screened.

Table 4-10: Gender mix in the screened population with Chi squared analysis confirming the significant difference in the proportion of males and females in the population with mucosal lesions but no significant difference between the groups of lesion.(= evaluated against the all lesions group, ^ = evaluated against the referred lesions subgroup, " = evaluated against the complex lesions subgroup and & = evaluated against all screened individuals)*

Total number of people screened		1111				
Males		645	58%			
Females		466	42%			
Total number of people with lesions		604				
Males	217	36%		0.000008[&]		
Females	387	64%				
All Lesions Detected		1438				
Males		544	38%			
Females		894	62%			
Referred Lesions		131				
Males	54	41%		0.537*		
Females	77	59%				
Complex Lesions		656				
Males	216	33%		0.288*	0.089^	
Females	440	67%				
Benign Lesions		782				
Males	328	42%		0.418*	0.056^	0.839"
Females	454	58%				

Paan chewing, in particular is known to be a cultural ritualistic trait amongst Bangladeshi women [88] although there was no difference in rates of paan usage reported between men and women in this population. In contrast smoking in the Bangladeshi population has been reported to be predominantly a male trait [23, 88] but again there was no difference, in our screened population. Pearson et. al (2001) [23] reported 53% of males smoking and just 5% of women although paan usage was more evenly distributed with 88% of women and 69% of men. This discrepancy maybe related to the brevity of data collection and not attempting to elucidate further details of paan and smoking habits in our study, in order not to interfere with the primary objective of OSCC screening. Additionally, the dentists were utilising interpreters to gather much of the information throughout the screening process with the Bangladeshi patients who often did not speak English. There may be translation issues in specifically explaining the difference between smoking tobacco and chewing tobacco or the distinction between areca nut containing compounds with and without tobacco. The other possibility is related to the screening methodology in Tower Hamlets, which at some locations focussed on encouraging women to attend by placing the mobile dental unit at specific sites such as Aden House Women's Association and actively recruiting participants utilising the staff at these community locations. This may account for the predominance of OMLs in women in the screened population as ladies with oral lesions may have congregated awaiting screening. There is some evidence of this effect in the data as when OMLs were present in women screened in Newham the average number of lesions per individual was 1.5 compared to 2.5 for men in Newham, 2.4 for women in Tower Hamlets and 2.7 for men in Tower Hamlets (Table 4-11). However, these numbers need to be interpreted with caution due to

the small sample sizes, especially in Newham where a just few people in different groups would skew the figures significantly.

Table 4-11: Proportions of individuals in which mucosal lesions where detected in each borough.

	Number of Patients	Number of Lesions	Lesions per Person
All Detected Lesions	Total 604	Total 1438	
Male	217 (36%)	544 (38%)	2.5
Female	387 (64%)	894 (62%)	2.3
Newham Individuals with Lesions	Total 141	Total 279	
Male	75 (53%)	165 (59%)	2.5
Female	66 (47%)	114 (41%)	1.5
Tower Hamlets Individuals with Lesions	Total 463	Total 1159	
Male	142 (31%)	379 (33%)	2.7
Female	321 (69%)	780 (67%)	2.4

4-3.12 Oral Cancer Risk Factors and Lesion Detection

A multiple logistic regression analysis was performed to determine any relationship between the presence of OMLs in the screened population and their risk factors for OSCC (Table 4-12). Individuals in the screened population who used paan were 3.43 times (CI: 1.18 - 5.31) more likely to have any OMLs than those who did not when controlling for the other risk factors of smoking, alcohol and age. Smokers were 2.52 times (CI: 1.02 - 4.54) more likely to present with OMLs than non-smokers controlling for paan and alcohol usage and age effects which would be expected as smoking is known to induce keratosis of the oral mucosa either by direct effect of heat or chemical irritation.

There was no statistically significant relationship with use of alcohol and the presence of all OMLs or with increasing age.

Table 4-12: Multiple logistic regression analysis showing the relationship between the presence of oral mucosal lesions and risk factors for oral cancer, in the screened population.

	Odds Ratio	95% Confidence Interval		Significance Level (p value)
		Low	High	
All Lesions (Total 1438)				
Paan	3.43	1.18	5.31	0.033
Smoking	2.52	1.02	4.54	0.041
Alcohol	1.04	0.73	1.91	0.268
Age	1.07	0.94	1.22	0.136
Benign Lesions (Total 782)				
Paan	1.02	0.79	1.19	0.491
Smoking	1.79	0.99	7.29	0.191
Alcohol	1.06	0.91	1.46	0.103
Age	1.12	0.97	1.19	0.144
Complex Lesions (Total 656)				
Paan	5.82	1.98	8.43	0.002
Smoking	3.59	1.12	4.47	0.027
Alcohol	1.14	0.92	1.71	0.092
Age	1.02	0.98	1.51	0.195

For benign lesions where the dentist is clinically certain there can be no malignancy there were no statistically significant relationships between any of the risk factors and the presence of these benign OMLs.

Complex lesions show the most prominent relationships between the risk factors and the presence of these difficult to diagnose OMLs. The use of paan is highly correlated with a 5.82 times (95% CI: 1.98 – 8.43) increase in complex OMLs controlling for smoking, alcohol and age related changes. Smoking is associated with a 3.59 times (95% CI: 1.12 – 4.47) elevated chance of presenting with a complex OML. As with the other groups there is no detectable relationship between alcohol usage and OML prevalence in the population or any age related changes in the multivariate regression analysis (Table 4-12).

4-3.13 Multiple Detected Lesions

In total 1438 OMLs were detected in the screened population of 1111 people. These lesions were all detected in just 604 of these individuals of which 188 (31%) had just 1 OML and the other 408 (69%) presented with multiple lesions (Table 4-13). The patients referred with suspected cancer totalled 66, consisting of 11 (17%) with 1 OML and 55 (83%) with multiple lesions. 656 OMLs were complex lesions and presented in 225 patients with again the majority of patients having multiple lesions (84%). The presence of 782 benign lesions in 379 patients was more evenly split with 40% presenting with 1 OML and 60% multiple OMLs. The presence of multiple OMLs in this population groups was also noted by Pearson et. al. (2001) [23] although the clinical significance was not discussed.

Table 4-13: The numbers of patients with one or multiple mucosal lesions in total and in the referred population as well as those with complex and benign lesions.

Total number of lesions	1438	
Number of patients with any detected lesions	604	
Patients with 1 lesion	188	31%
Patients with >1 lesions	416	69%
Referred lesions in total	131	
Number of referred patients	66	
Refereed patients with 1 lesion	11	17%
Referred patients with >1 lesions	55	83%
Complex lesions in total	656	
Number of patients with complex lesions	225	
Patients with 1 lesion	35	16%
Patients with >1 lesions	190	84%
Benign lesions in total	782	
Number of patients with benign lesions	379	
Patients with 1 lesion	153	40%
Patients with >1 lesions	226	60%

4-4.01 DISCUSSION

4-4.02 The High Prevalence of OMLs in the Screened Population

The presence of large numbers and a wide variety of OMLs in the screened population for OSCC is likely to be the cause of misinterpretation and resultant reduced sensitivity of screening by direct visual examination.

In total 1438 OMLs were detected in 604 (54%) individuals in the screened population of 1111 individuals (Table 4-11). This is considerably higher than the 40% prevalence reported by Pearson et. al. (2001) [23] in a similar population although this can be explained by the targeting of high-risk individuals rather than simply observing patients in a general medical practice waiting room. Also our reporting criteria included common lesions such as frictional keratosis (36%) and paan staining (36%) (Table 4-06) which appear to be not often reported in other studies [23-25]. This could be because they are so widespread, in this population, as to be considered normal variations for a population with high levels of areca nut compound and tobacco usage. Additionally, paan staining is not a specific clinical lesion in the conventional terminology so maybe overlooked when assessing OMLs. These lesions are clinically important because they complicate the assessment of OSCC by direct visual examination. Frictional keratosis presents as a white patch which can only be excluded from a diagnosis of leukoplakia by a careful history and examination to determine the specific cause of frictional trauma. Therefore, if no clear cause can be elicited, keratosis, must be classified as leukoplakia and in a high-risk individual, biopsied to exclude dysplasia [15]. Paan staining by definition occurs in high-risk individuals due to the use of areca nut containing compounds and its red-brown discolouration of the mucosa may mask

or alter the appearance of other OMLs, necessitating careful drying, washing and debridement of the mucosal surface for adequate visual examination. Paan staining and frictional keratosis are therefore significant issues that directly affect OSCC detection by visual examination and cannot be ignored when evaluating the role of OMLs in screening efficacy for OSCC.

As well as the high prevalence of OMLs, 32 different types of mucosal lesion were detected in the screened population (Table 4-06). 134 lesions were conventionally described as PMDs (9.3%) including leukoplakia representing (4.1%), OSF (0.5%), erythroleukoplakia (0.8%) and erythroplakia (0.3%). Other rare OMLs such as Fordyce spots and herpetiform aphthous ulceration were also detected which also serve to complicate the assessment of the oral mucosa. Therefore, the high prevalence and wide variety of OMLs in the screened population confirms the difficulty reported by examiners in screening for OSCC by direct visual examination the Tower Hamlets and Newham populations.

4-4.03 The Value of Classifying OMLs as Complex or Benign

OMLs have conventionally been reported in the literature as benign or PMDs or OSCC. PMDs have a broad definition of “all clinical presentations that carry a risk of cancer” [15]. Discussions are often limited to leukoplakia and erythroplakia such as in the WHO group review by Napier et. al. (2008) [29] although the broader description may be more helpful clinically especially in high-risk population where non-leukoplakia PMDs, such as OSF, are common. This confusion may have arisen because the definition of PMDs as “all clinical presentations that carry a risk of cancer” is not specifically stated in the report by Warnakulauriya et. al. (2007)

unlike their clear definition of leukoplakia. The PMD definition occurs in a discussion regarding the lack of value in the previous WHO terminology differentiating between ‘lesions’ and ‘conditions’.

Of 1438 oral mucosal lesions detected in the screened population 54% were clinically diagnosed as benign and no patients with these lesions were referred (Figure 4-03). This implies the clinicians can readily exclude malignancy even if risk factors are prevalent which is consistent with the high specificity reported for direct visual assessment for OSCC [62]. Of the 46% of lesions that were not definitively benign only a proportion were referred (Figure 4-03, Table 4-07 and Table 4-08) as either conventionally described PMDs or suspected malignancy. The number of clinically ‘not-benign but also not definitively malignancy’ OMLs which also are not usually described as PMDs indicates the need for another term such as complex oral mucosal lesions. An important example of an OML that falls between the current classification is paan staining or paan associated mucositis whereby it’s presence alone indicates risk factors for OSCC and the staining may also obscure malignant mucosal changes. Therefore, complex lesions would be a screening-specific term used in direct visual assessment of the oral mucosa relating to any abnormality that was not definitively benign. Complex lesions would include PMDs and any other mucosal abnormality that the screening dentist could not assign a benign diagnosis to and therefore, in high-risk individuals, could not be certain was not malignancy. Complex is a better description than ‘suspicious’ for a lesion as it indicates the uncertainty of the visual assessment process without directly indicating the lesion is malignant or potentially malignant. Therefore,

complex lesions could be watched or acted on whilst all suspicious lesions require action.

Only 17 of 51 (33%) screening diagnosis could be considered accurate when definitive diagnosis is known (Table 4-07 and 4-08). This is predominantly because it is impossible to differentiate benign hyperkeratosis in clinically evident leukoplakia from those lesions with dysplasia on the basis of a direct visual examination. Therefore, detection of leukoplakia by direct visual examination will inevitably have very low sensitivity and specificity without adjuncts to aid diagnosis. The screening diagnoses were most accurate for the more clinically distinct PMDs such as OSF where all 6 cases were accurately reported and LP or lichenoid reactions where all 4 patients were also accurately reported. Of the patients eventually discharged from further follow-up, indicating no significant risk of malignancy, there was range of screening clinical diagnosis including leukoplakia, erythroleukoplakia and even erythroplakia confirming the difficulty a screening dentist has in making a clinical diagnosis with PMDs.

Amongst the complex clinical lesions detected, Figure 4-04 shows that 100% of erythroleukoplakias, erythroplakias and ulcers suspected to be OSCC were referred to the tertiary referral centre. 93% of detected leukoplakias were referred due to the presence of significant risk factors such as tobacco and paan usage with the remainder detected in low risk individuals and therefore classified as routine referrals and inappropriate for a suspected cancer pathway. 67% of lichenoid reactions and 12% of reticular LP were also referred but always when present in conjunction with other lesions more indicative of OSCC, such as

erythroleukoplakia. Six out of 7 patients with OSF were referred because of the presence of associated lesions of concern and the other was also already under the care of the hospital for their condition. Paan staining itself was not an indication for referral but 11% of individuals also had other referable lesions. Together, this suggests that the group of complex clinical lesions where the dentist's clinical judgment is required to determine whether referral is appropriate comprises leukoplakia, lichenoid reactions, LP, OSF and paan staining either because the lesion may itself be malignant or because it maybe associated with other PMDs. These are the lesions that complicate referral for OSCC screening dentists and therefore are most likely responsible for the low sensitivity and PPV of direct visual screening for OSCC.

As well as the clinical diagnosis the other factor in the decision to classify lesions as complex or benign is their location in the oral cavity. Mucosal lesions were detected throughout the oral cavity (Table 4-09) with the largest proportion (48%) on the buccal mucosa including the buccal sulcus. This is consistent with a significant proportion of the screened population using paan, which results in traumatic keratosis at the site of usage, and therefore the buccal area was also the largest single site for the complex (36%) and referred (35%) OMLs. The buccal mucosa is also often the site of frictional keratosis related to the occlusion and thus was also the single commonest site for benign mucosal lesions. Pearson et. al. (2001) [23] also reported the majority of OMLs were detected on the buccal mucosa in their study of Bangladeshi medical care users, confirming the likely aetiology. There were no clinically benign lesions reported on the FOM or ventral tongue, which are conventionally high-risk sites for OSCC (Figure 4-05). This

would indicate the dentists were unable to exclude malignancy in any lesions at these sites whilst conversely there were no referred lesions and very few complex lesions on the dorsum of the tongue consistent with this being a very rare site for OSCC to develop.

Taken together this data suggests that the screening diagnosis of a benign or complex lesion specifically relates to both the screening dentists clinical diagnosis and the high-risk site of the OML suggesting this terminology is more accurate in reflecting the clinical reality of OSCC screening by visual assessment of the oral mucosa than the use of the term 'potentially malignant disorder'.

4-4.04 Significance of the Multiple OMLs in the Screened Patients

It has been reported in previous studies of the Bangladeshi population of Tower Hamlets that patients often present with multiple OMLs [23] but the significance of this has not been evaluated. The screening dentists in this study also reported that difficulties in diagnosing patients with suspicious lesions occurred due to the presence of multiple oral mucosal abnormalities. Therefore, it would be expected to see more patients in the referred population with multiple OMLs than the general screened population. The proportions of patients presenting with 1 OML or multiple OMLs are significantly different between the referred group and the total group of people with OMLs (Figure 4-06). Comparison of proportions of patients with multiple mucosal lesions shows the composition of the referred patient's group is also different to the group of patient's with benign lesions but similar to the complex lesions group. This could simply be because the referred patients, by definition, are a sub-group of the complex lesions group but as they only comprise

about 10% (66 out of 656) of this group, the indication is of some correlation between multiple lesions and referral. This is supported by the benign lesion group (where there were no referrals) having statistically significantly fewer patients with multiple lesions. The relationship is complex because any one lesion can initiate a referral, if suspicious enough to the clinician, but it would also seem reasonable that more OMLs would increase the screening clinician's level of uncertainty and concern, especially if the lesions are not obviously benign. To add some degree of triangulation validity to this analysis, as well as the benign lesions group being significantly different in proportion of patient's with multiple OMLs, to the referred patient group and the complex lesions group, it is statistically similar to the whole population of patients with OMLs (Figure 4-06).

Overall it would appear that the screening dentists were referring higher proportions of patients with multiple OMLs, specifically if they were complex OMLs. This could indicate the dentists were struggling to make a definitive diagnosis of no OSCC by visual assessment of the oral mucosa alone, when there were multiple complex OMLs present. Multiple benign OMLs did not appear to cause this diagnostic dilemma.

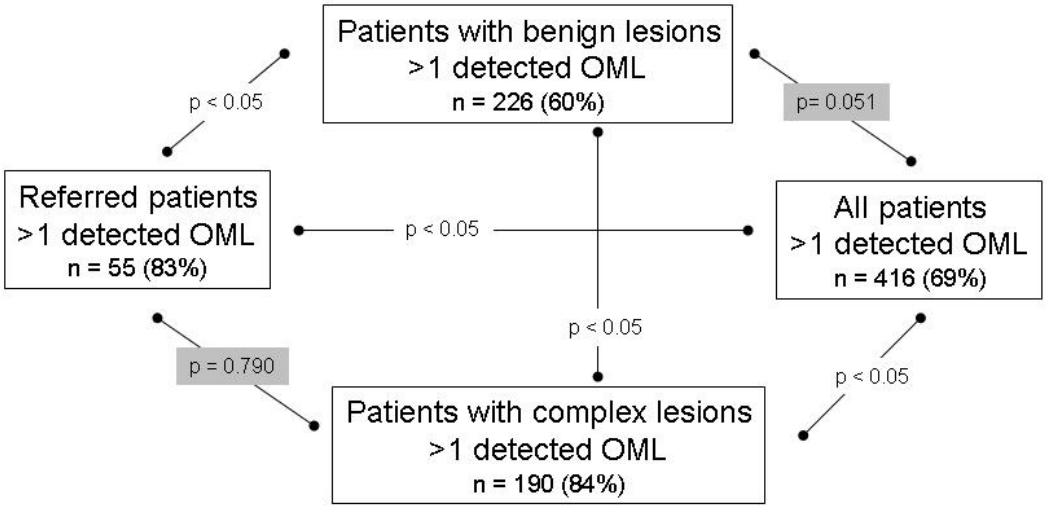


Figure 4-06: Chi-squared comparison of proportions of patients with multiple mucosal lesions shows the composition of the referred patients group is similar to the complex lesions group and different to all other groups. The benign lesions group is significantly different in proportion of patients with multiple lesions to the referred patient group and the complex lesions group but similar to the whole population of patients with multiple OMLs.

4-4.05 Oral Cancer Risk Factor Correlation with Lesion Detection

Individuals in the screened population who used paan were 3.43 times (CI: 1.18 - 5.31) more likely to have any OMLs than those who did not when controlling for the other risk factors of smoking, alcohol and age (Table 4-12). This would be consistent with paan usage having substantial staining effects on the oral mucosa presenting as visibly detectable OMLs most notably at the site of paan usage. Smokers were 2.52 times (CI: 1.02 - 4.54) more likely to present with OMLs than non-smokers controlling for paan and alcohol usage and age effects which would be expected as smoking is known to induce keratosis of the oral mucosa either by direct effect of heat or chemical irritation. Use of tobacco and areca nut compounds resulting in increasing numbers of OMLs is consistent with evidence that these risk habits are directly related to the presence of leukoplakia as shown by Gupta et. al (1992) [124] in a large scale intervention study where stopping tobacco usage also drastically reduced the incidence of leukoplakia.

There was no statistically significant relationship with use of alcohol and the presence of all OMLs. An explanation could be the small sample size for alcohol users as only 12% of those screened in Tower Hamlets admitted drinking alcohol. It is also possible that alcohol use is infrequent and minimal, in this population, as no attempt was made to quantify units or regularity of use. Alternatively alcohol use may not result in visible OMLs in the same way as paan or smoking as there is no frictional or heat component to alcohol in the oral cavity.

Age has previously been linked with the presence of OMLs in the Bangladeshi population [23] yet our multiple logistic regression analysis showed no association (Table 4-12). The lack of a relationship in the screened population maybe related to the screened population's relatively low mean age 42.3 (SD 15.9 years) for Tower Hamlets (Table 3-03). This would imply relatively fewer older people and the resultant small sample size would directly affect the ability to show a statistically significant relationship with the older age ranges. It is also known that logistic regression algorithms are systematically inaccurate for odds ratios when the sample size is less than 500 although conversely the degree of inaccuracy is often much lower than the standard error of the estimate and therefore potentially insignificant [125]. The other possibility is that age presents as a compound risk with increasing time of the other behavioural risk factors and the multiple logistic regression analysis methodology controls for the other risk factors thereby negating this effect. To evaluate this, a univariate linear regression for one continuous exposure variable was carried out on the 1111 screened individuals with the single outcome variable being the presence of any OMLs and no controls for any of the other risk factors (Table 4-14).

Table 4-14: Linear regression analysis to determine the relationship between age and the presence of OMLs in the screened population.

	Odds Ratio	95% Confidence Interval		Significance Level (p value)
		Low	High	
All Lesions (Total 1438)				
Age	1.01	1.00	1.03	0.011
Benign Lesions (Total 782)				
Age	1.11	0.97	1.17	0.217
Complex Lesions (Total 656)				
Age	1.04	1.01	1.14	0.041

The interpretation of the linear regression analysis would be that for every one year increase in age, the odds of developing a OMLs increase by a factor of 1.01 (95% CI: 1.00 -1.03) or by about 1% each year. The relationship shown by linear regression but not by multiple regression controlling for paan usage and smoking suggests age presents a cumulative effect of the other risk factors but in itself is not a risk factor for the development of OMLs. Older patients essentially have more time for pathological features to develop from paan usage and smoking and the incidence of OMLs rises because of an inability to repair this pathological damage.

For benign lesions where the dentist is clinically certain there can be no malignancy there were no statistically significant relationships between risk factors and the presence of OMLs. This is consistent with all paan related lesions being of some concern to the dentist and therefore not classified as benign hence the lack of a relationship with paan usage unlike for all OMLs together. The bulk of the benign lesions were frictional keratosis (71%) (Table 4-06) and they are

predominantly on the buccal mucosa (Table 4-09) indicating an occlusal trauma aetiology. This would explain the lack of a relationship with smoking and it is also feasible that smoking being a risk factor for OSCC caused the dentist to classify any lesions in smokers as complex for that reason alone.

Complex lesions show the most prominent relationships between the risk factors and the presence of these difficult to diagnose OMLs. The paan usage related increase is highly statistically significant with a 5.82 times (95% CI: 1.98 – 8.43) increase in complex OMLs controlling for smoking, alcohol and age related changes. This might be expected because of the visible nature of paan related staining of the oral mucosa alerting the dentist to the OSCC risk. Smoking is associated with a 3.59 times (95% CI: 1.12 – 4.47) elevated chance of presenting with a complex OML whilst controlling for paan or alcohol usage and age related changes. As with the other groups there is no detectable relationship between alcohol usage and OML prevalence in the population and any age related changes may again be being masked by the statistical approach. Linear logistic regression analysis of the complex lesion sub-population confirms that age is correlated with the presence of OMLs (Table 4-14) but the effect is again likely to be related to the other risk factors as it is not apparent when controlling for paan, smoking and alcohol usage (Table 4-12). Also the age effect is more evident than with the total population of OMLs as the odds ratio is 1.04 (95% CI: 1.01 – 1.14) suggesting a 4% increase in OMLs per year compared to 1% for all OMLs (Table 4-14). This would suggest the risk factors have a more pronounced age effect on people with complex lesions than benign or all OMLs supporting the increased risk status of this group determined clinically by the dentists.

4-5.01 CONCLUSION

OMLs are highly prevalent amongst patients targeted for high risk screening in the South Asian populations of Tower Hamlets and Newham. The OMLs detected can be classified as clearly benign or more complex and problematic lesions which may adversely affect the screening dentist's ability to diagnose suspected OSCC accurately. The difficult complex lesions are predominantly leukoplakias as the more distinct clinical presentation of PMDs such as OSF and LP is more readily diagnosed. The presence of multiple complex OMLs in individual patients further hinders the dentist's ability to screen accurately for OSCC potentially resulting in more false positive outcomes. Furthermore, the lifestyle risk factors for OSCC that are prevalent in the South Asian population, paan usage and smoking, increase the prevalence of these complex OMLs in a time dependent manner.

Taken together this indicates that targeted screening for OSCC in South Asian populations by direct visual assessment is always likely to result in high levels of false positives because of the presence of other OMLs. Therefore, an effective screening protocol for South Asian populations in the UK could not be performed by direct visual assessment alone necessitating diagnostic aids to increase screening sensitivity.

CHAPTER FIVE

IMMUNOHISTOCHEMICAL ANALYSIS OF KERATIN EXPRESSION IN FRESH FROZEN TISSUE SPECIMENS OF ORAL SUBMUCOUS FIBROSIS

Chapter 5

Immunohistochemical Analysis of Keratin Expression in Fresh Frozen Tissue Specimens of Oral Submucous Fibrosis

5-1.01 INTRODUCTION

From experience of screening in the Tower Hamlets and Newham South Asian populations we have observed two major issues that need resolving before a viable targeted screening programme can be contemplated. Firstly the issue of non-attendance of screen positive individuals at referral, resulting in an inability to accurately evaluate screening outcomes and the potentially more important, but completely unquantifiable, effect on the screened individual. Despite all possible measures to prevent this 21 out of 86 (24%) of all screen positive individuals from the screened population did not comply with referral which may be an inherent property of this population as referral compliance in UK studies is usually reported approaching 100% yet South Asian based studies are consistent with our findings [62, 109]. The second major issue is the high prevalence of OMLs in this population and in particular those complex lesions that cannot clinically be diagnosed as benign and the consequential difficulty in determining which lesions require further investigation for malignancy. These two issues are clearly inter-related as the difficulty in diagnosis leads to more false positive outcomes from screening activity with resultant larger numbers of screen positive individuals referred and subsequently failing to attend for definitive diagnosis. The simplest solution is to undertake the definitive diagnosis at the time of screening.

5-1.02 Definitive Diagnosis of Oral Mucosal Lesions

The standard process for definitive diagnosis of OMLs is a combination of gross (macroscopic) examination followed by histologic (microscopic) examination of a tissue section which may be aided by evaluation of the molecular properties of the lesion by immunohistochemistry of the tissue section. During cancer resection surgery these stages are often carried out in an immediate manner utilising frozen section histology to evaluate resection margins before surgical reconstruction is attempted.

A variant of this ‘intra-operative pathology support’ approach would be relatively straightforward on a mobile dental unit which could house facilities to immediately biopsy and snap freeze samples that would be couriered to a pathology laboratory for sectioning, staining and diagnosis. The screen positive patient could have a definitive diagnosis within a few hours, as to whether they actually had oral cancer or not.

PMDs are more problematic as although histology is valuable for diagnosis it is their potential to become OSCC that is the most important concern. Our screening outcomes suggest some PMDs, such as LP and lichenoid reactions, can be readily diagnosed by direct visual examination and this would be enough to indicate the patient with relevant risk factors cannot be discharged from further follow-up, which is the most appropriate soft gold standard for a positive screening outcome in the UK. Leukoplakia was a more difficult diagnostic decision for the screening dentist, especially with the paan staining commonly found in high-risk South Asian populations such that the majority of lesions referred as leukoplakia

were actually benign hyperkeratosis (Table 4-07 and Table 4-08). The presence of dysplastic change is the most widely used and reliable indicator of malignant potential in leukoplakia [33] and this can be assessed on fresh frozen sections in a similar manner to that used to determine clear resection margins. For the soft gold standard outcome the presence or absence of dysplasia would be appropriate for a positive result. From our experience an average of 2-3 individuals were screened positive on each screening day and would require intra-operative pathology support for definitive diagnosis which may be feasible within existing healthcare services.

In relation to South Asian populations, of particular concern is OSF because of the significant potential for malignant conversion (7-26%) [39] as well as the clinical complications associated with the fibrosis itself. OSF presents a specific problem in screening for OSCC because of its direct association with areca nut compounds [41] found in paan. However, OSF only occurs in a small proportion of areca compound users, such as the 7 out of 568 (1.2%), reported in our sample, suggesting that despite the association with areca it's specific aetiology is complex and not clearly understood. Paan staining itself was a significant issue reported by the dentists when attempting direct visual assessment of the oral mucosa during the screening programme. Therefore, histological methods to aid diagnosis of OSF would be valuable for improving the sensitivity of OSCC screening in high-risk South Asian populations.

5-1.03 Immunohistochemistry

Immunohistochemistry (IHC) is the process of detecting antigens in the cells of a tissue section by utilising the specificity of antibody binding to those antigens (Figure 5-01). IHC shows exactly where the antigen is located within the tissue examined. Its major disadvantage is that, unlike immunoblotting techniques where staining is checked against a molecular weight ladder, it is impossible to show in IHC that the staining definitively corresponds to the protein of interest. Therefore, the antibodies used must be well-validated in Western Blots or similar procedures to validate IHC staining patterns.

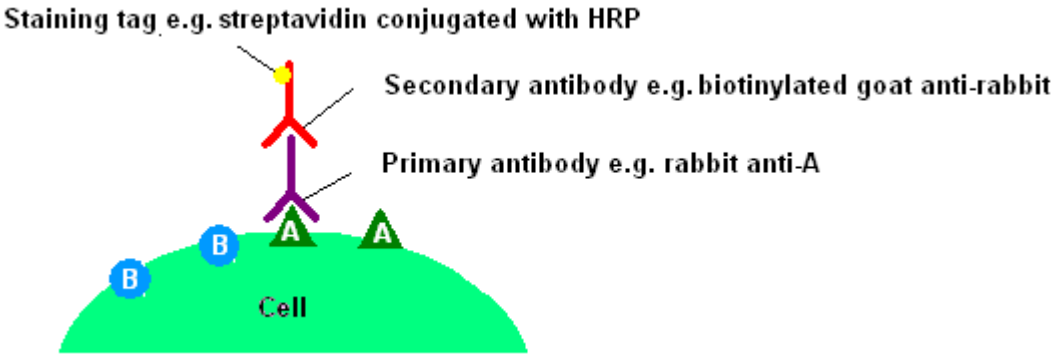


Figure 5-01: Two layer indirect immunohistochemical staining for antigen A, using a primary anti-A antibody and a secondary antibody raised against the primary. The secondary is also labelled with biotin which binds streptavidin. The streptavidin is conjugated with Horse Radish Peroxidase (HRP) which oxidises 3,3'-Diaminobenzidine (DAB) to a visible brown colour.

Various different systems exist for IHC with the most common based on the avidin-biotin system [126]. The primary antibody is raised against the antigen of interest (e.g. 'A' in Figure 5-01) in a species that will not cross-react with human, such as rabbit. The primary then binds specifically to antigen A and any excess is washed away leaving only specific bound antigen–antibody complex. A secondary antibody raised against the species of the primary (i.e. rabbit) then binds the

primary-antigen A complex. The secondary is labelled with biotin reporter molecules which have a very high affinity for streptavidin. Streptavidin itself is conjugated with the enzyme horse radish peroxidise (HRP). HRP can be made visible using a substrate that, when oxidized by HRP using hydrogen peroxide as the oxidizing agent, produces a characteristic colour. 3,3'-Diaminobenzidine (DAB) is one such substrate that produces a clear brown colouration of the specifically stained areas of tissue. As streptavidin has four binding sites for biotin and multiple biotin molecules label the secondary antibody this amplification system allows for maximum sensitivity of detection.

5-1.04 Fresh Frozen and Formalin Fixed Tissue Sections

Fresh frozen tissue specimens ideally require a source of extreme cold for snap freezing to preserve cytoarchitecture and molecular markers as well as allowing cryosectioning for IHC. For this reason fresh frozen sections are considerably more difficult to handle than formalin fixed paraffin embedded tissue where the fixation process minimises degradation of the tissue. Unfortunately the fixation process is also known to mask antigens and interfere with antibody binding. This is thought to be due to formaldehyde associated methylene bridge cross-linking affecting the tertiary and quaternary structures of proteins [127]. To permit antibody binding a process of antigen retrieval is usually required and commonly involves high temperature and a citrate or ethylenediaminetetraacetic acid (EDTA) antigen retrieval buffer. The antigen retrieval process is known to compromise tissue morphology [126] and in combination with formalin fixation is a lengthy procedure unsuitable for an intra-operative pathology support services.

5-1.05 Normal Oral Mucosa

The oral cavity is lined by a mucous membrane consisting of epithelium overlying a collagenous connective tissue lamina propria. The oral mucosa predominantly serves as a lining and protective barrier to the external environment. Histologically and functionally oral mucosa is classified as masticatory, lining or specialised. Lining mucosa makes up 60% of all oral mucosa and covers the labial and lingual sulcus, floor of mouth, soft palate and buccal mucosa. It is flexible to allow unrestricted function of the underlying musculature and comprises non-keratinised oral epithelium overlying a loose lamina propria and fatty or glandular submucosa. The masticatory mucosa of the hard palate and gingivae comprises 25% of the oral mucosa and is designed to withstand the abrasion and pounding of a food bolus during mastication. The epithelium of masticatory mucosa is keratinised and tightly bound to a dense connective tissue by large numbers of deep rete ridges and the lamina propria itself is attached to bone with no intervening submucosa. Specialised oral mucosa involved in taste sensation is found on the dorsum of the tongue and the lip mucosa also has an important sensory function.

5-1.06 Oral Epithelium

The oral epithelium is separated from the lamina propria by a 1-2μm thick basement membrane (BM) and constitutes the primary barrier to the external environment. Oral epithelium is a stratified squamous structure made up of layers of tightly adhered keratinocytes. The surface layer is constantly shed as a protective mechanism necessitating that the epithelium maintains its structural integrity by a continuous process of keratinocyte renewal whereby cells produced in the deepest layers migrate towards the surface whilst undergoing a process of

terminal differentiation, to replace those that are shed. Therefore, two distinct populations of epithelial cells can be described in the epithelium: a progenitor population predominantly located in the basal layer which undergoes cell division to produce new keratinocytes and a maturing cell population undergoing sequential terminal differentiation to replenish the surface layer. The progenitor population comprises a small number of adult stem cells and a larger population of transit-amplifying (TA) cells. Stem cells possess unlimited self-renewal capacity but rarely undergo cell division. A stem cell can either divide symmetrically to produce two identical stem cells or divide asymmetrically to regenerate and produce a TA cell [128]. The TA cells migrate laterally to populate the basal layer and undergo limited rounds of rapid cell division to increase the TA pool of cells, and then become committed to undergo terminal differentiation in the suprabasal layers. This mechanism allows a large number of TA cells to be produced from a single stem cell division thus protecting the stem cells from acquiring genetic lesions because DNA is most at risk from environmental mutagenic agents whilst cells are actively dividing. The maturing cell population follows two distinct terminal differentiation lineages which results in either a keratinised or non-keratinised oral epithelium.

5-1.07 Keratinised and Non-Keratinised Epithelium

Keratinisation produces a tough, impermeable surface layer to the oral epithelium that is consequently less flexible than non-keratinised epithelium. Keratinised oral epithelium comprises cuboidal basal cells adjacent to the basement membrane and below several layers of larger elliptical keratinocytes in the prickle cell layer. The basal and prickle cell layers constitute over half the thickness of keratinised

epithelium and lie beneath the granular layer of flattened keratinocytes containing keratohyalin granules. The surface layer or stratum corneum consists of flattened squames which have no cellular organelles and are composed entirely of impermeable cross-linked keratin filaments (Figure 5-02). This differentiation lineage is referred to as ortho-keratinisation whilst a stratum corneum of flattened keratinocytes with pyknotic nuclei occurs in para-keratinisation and is a normal variation.

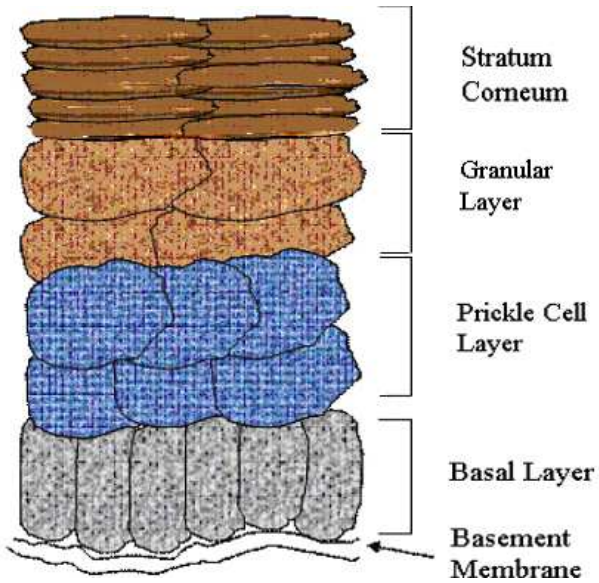


Figure 5-02: Schematic representation of the appearance of keratinised oral epithelium.

Non-keratinised oral epithelium is usually thicker than keratinised epithelium with the buccal epithelium often over 500μm from BM to surface. The basal and prickle cells resemble those of keratinised epithelium whilst the outer layers are arbitrarily divided into an intermediate zone and a surface zone with no significant difference in the appearance of the keratinocytes (Figure 5-03).

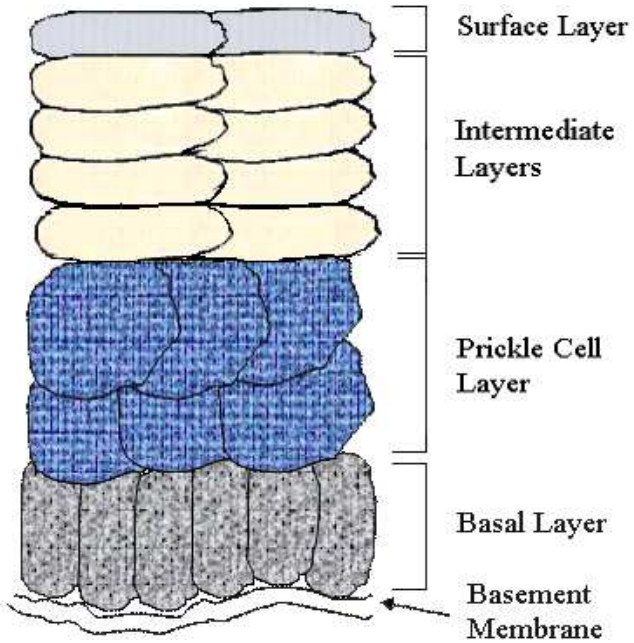


Figure 5-03: Schematic representation of the appearance of non-keratinised oral epithelium.

5-1.08 Non-keratinocytes in the Epithelium

Up to 10% of the cell population in oral epithelium are non-keratinocytes. In H+E sections these appear as ‘clear cells’ having a clear halo around their nuclei due to histologic processing resulting in contraction of the cytoplasm as it lacks the insoluble keratin cytoskeleton of the keratinocytes. Non-keratinocytes comprise pigment producing melanocytes and sensory Merkel cells in the basal layer of the epithelium as well as a few inflammatory Langerhans’ cells and lymphocytes that migrate in and out of the normal epithelium.

5-1.09 The Lamina Propria

The lamina propria consists of cells, blood vessels, neural components and fibres embedded in an amorphous extracellular matrix. The lamina propria is divided into two layers: the superficial papillary layer associated with the epithelial rete ridges and the reticular layer between the papillary layer and the deeper structures. The

reticular layer contains thick bundles of collagen fibres arranged parallel to the surface plane whilst the papillary layer is made up of finer loosely arranged collagen fibres and capillary loops. The principal cells found throughout the lamina propria are fibroblasts which are involved in the production and turnover of collagen, elastin and fibronectin as well as the proteoglycan rich extracellular matrix. Other cell types in the lamina propria include those involved in inflammation and host responses such as histiocytes, macrophages, neutrophil polymorphs, lymphocytes, plasma cells and mast cells. Endothelial cells are found lining vascular channels throughout the lamina propria.

5-1.10 OSF Histological Features

The characteristic pathological change in OSF is progressive collagenous fibrosis and hyalanisation of the sub-epithelial connective tissue and histological classification of OSF severity is based entirely on the extent of fibrosis and degree of inflammatory infiltration [129]. (Figure 5-04)

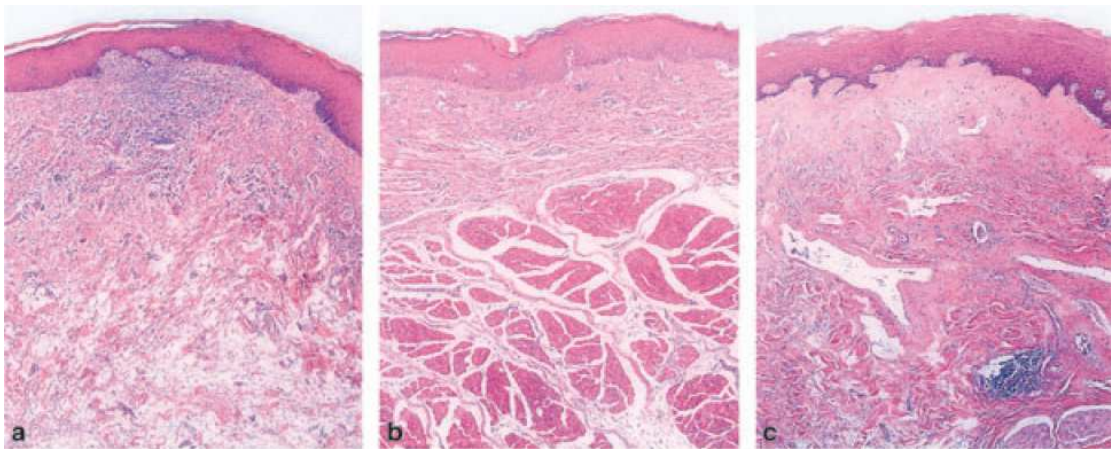


Figure 5-04: The histological presentation of OSF. (a) Early – subepithelial inflammatory infiltrate (neutrophil polymorphs, lymphocytes and eosinophils) with dilated and congested vessels producing a marked oedema but no significant fibrosis of the connective tissue. (b) Intermediate – subepithelial fibrosis with early hylanisation characterised by thickened collagen bundles and moderate numbers of fibroblasts and chronic inflammatory cells (lymphocytes and plasma cells). (c) Advanced – extensive hyalinsation of the subepithelial connective tissue with fibrosis extending into the underlying muscle or adipose tissue and little inflammatory infiltrate or oedema due to decreased vascularity of the connective tissue. (Adapted from Utsunomiya et al. 2005 [16])

5-1.11 OSF Related Epithelial Changes

Although OSF is believed to be a connective tissue disorder certain epithelial changes have been noted ever since the first histological evaluations. Dysplasia has been reported in 26% of OSF lesions [130], which is consistent with the reported rates of malignant transformation to OSCC of 7-26% [39]. Keratinising metaplasia is present in 67% of cases [131] and is believed to be due to direct mechanical trauma from the coarse fibres of the areca nut. Thinning of the epithelium occurs in 87% of OSF lesions [131] and was attributed to epithelial atrophy as a result of reduced submucosal vasculature and consequent lack of nutrient provision. More recently Rajendran et al. (2004) [132] have shown there is no increased keratinocyte cell death, either by apoptosis or necrosis, which is a prerequisite for epithelial atrophy and therefore suggested that reduced keratinocyte proliferation and epithelial hypoplasia as the mechanism for epithelial thinning in OSF. The other epithelial abnormality often described in OSF histological sections is pigment incontinence from melanocytes embedded in the basal layer of the epithelium.

Despite these consistent epithelial abnormalities reported in OSF, it is conventionally regarded as a connective tissue disorder with the epithelial changes a consequence of the underlying pathology. Even malignant transformation, the most significant and epithelial complication of OSF, is considered to occur because of the build-up of carcinogenic stimuli within the avascular sub-epithelial connective tissue. From the literature on other oral mucosal lesions and cutaneous fibrotic disorders it is apparent that gross epithelial changes often involve alterations within the individual keratinocytes and these keratinocytes are intimately involved in interactions with their surroundings [133].

Understandably, there is no data in the literature on these epithelial interactions in OSF.

5-1.12 Keratins

Keratinocytes display two structures that distinguish them as epithelial, one is the intercellular desmosome responsible for cellular cohesion and the other is their cytoskeletal filaments. The keratinocyte cytoskeleton comprises a class of intermediate filament forming proteins termed keratins, that as well as providing mechanical support for the cell are involved in: cytoarchitecture, stress responses, regulation of signaling pathways towards apoptosis and protein synthesis, and organelle/vesicle distribution [18].

5-1.13 The Moll Nomenclature

Moll et. al (1982) [134] provided the first attempt at a comprehensive keratin nomenclature by grouping the basic-neutral type II keratins as K1-8 and the acidic type I keratins as K9-19. The subsequent discovery and characterisation of further type I and type II keratins and the “hard” hair and nail keratins necessitated a new systematic nomenclature which could incorporate the 54 functional keratin genes demonstrated by analysis of the complete human genome. 28 type I and 26 type II keratin genes form two clusters of 27 genes on chromosomes 17q21.2 and 12q13.13 with the gene for type I K18 being located in the type II keratin gene domain of chromosome 12 [135] (Figure 5-05).

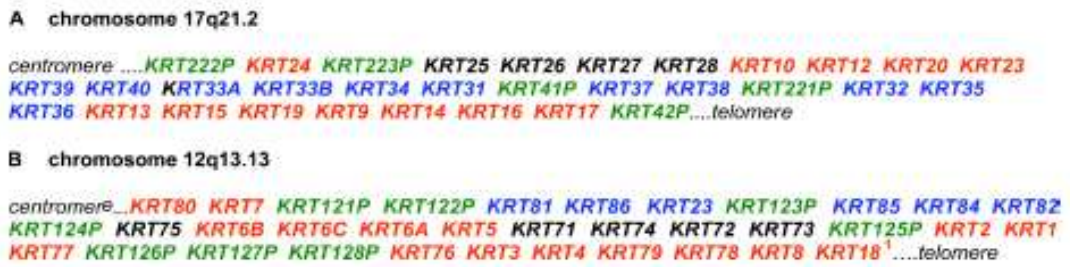


Figure 5-05: Human keratin gene domains. (A) type I keratins and (B) type I., (Red) epithelial keratin genes, (Black) hair follicle specific epithelial keratin genes, (Blue) hair keratin genes and (Green) keratin psuedogenes. [1] KRT18 is a type I keratin gene.
(From: Schweizer et al 2006 [17])

5-1.14 The New Consensus Nomenclature

In 2006 Schweizer et. al. [17] reported a new consensus nomenclature for keratins updating the Moll designations whilst retaining the HGNC gene designation scheme (Table 5-01, 5-02 and 5-03). This classification also does away with the alternative name ‘cytokeratin’ with preference for just ‘keratin’.

Table 5-01: Numbering scheme of keratin groups (From: Schweizer et al. 2006 [17])

Category	Number range
Human type I epithelial keratins	9–28
Human type I hair keratins	31–40
Nonhuman type I epithelial and hair keratins	41–70
Human type II epithelial keratins	1–8 and 71–80
Human type II hair keratins	81–86
Nonhuman type II epithelial and hair keratins	87–120
Type II keratin pseudogene	121–220
Type I keratin pseudogenes	221 →

Table 5-02: The Moll classification and the new consensus nomenclature of Type I keratins. (Adapted from: Schweizer et al. 2006 [17])

Current protein designation	Current gene designation	New protein designation	New gene designation
Human epithelial keratins			
K9	*KRT9*	K9	*KRT9*
K10	*KRT10*	K10	*KRT10*
K12	*KRT12*	K12	*KRT12*
K13	*KRT13*	K13	*KRT13*
K14	*KRT14*	K14	*KRT14*
K15	*KRT15*	K15	*KRT15*
K16	*KRT16*	K16	*KRT16*
K17	*KRT17*	K17	KRT17
K18	*KRT18*	K18	*KRT18*
K19	*KRT19*	K19	*KRT19*
K20	*KRT20*	K20	*KRT20*
K21 rat	no designation	—	—
K23	*KRT23*	K23	*KRT23*
K24	*KRT24*	K24	*KRT24*
K25irs1[b], K10C[c], hIRSa1[d]	*KRT25A*	K25	KRT25
K25irs2[b], K10D[c]	*KRT25B*	K26	*KRT26*
K25irs3[b], K10B[c], hIRSa3.1[d]	*KRT25C*	K27	*KRT27*
K25irs4[b], hIRSa2[d]	*KRT25D*	K28	*KRT28*
Human hair keratins			
Ha1	*KRTHA1*	K31	*KRT31*
Ha2	*KRTHA2*	K32	*KRT32*
Ha3-I	*KRTHA3A*	K33a	*KRT33A*
Ha3-II	*KRTHA3B*	K33b	*KRT33B*
Ha4	*KRTHA4*	K34	*KRT34*
Ha5	*KRTHA5*	K35	*KRT35*
Ha6	*KRTHA6*	K36	*KRT36*
Ha7	*KRTHA7*	K37	*KRT37*
Ha8	*KRTHA8*	K38	*KRT38*
		K39	*KRT39*
		K40	*KRT40*
Nonhuman hair/ epithelial keratins			
	φhHaA , *Ka34P* man	K41 chimp, gorilla	*KRT41* chimp, gorilla *KRT41P* man
K17n mouse, rat	no designation	K42 mouse, rat	*KRT42* mouse, rat *KRT42P* man
Human keratin pseudogenes			
	φhHaB		*KRT221P*
	φKRTJ[b], *Ka21P*		*KRT222P*
	φKRTJ[b], *Ka37P*		*KRT223P*

Table 5-03: The Moll classification and the new consensus nomenclature of Type II keratins. (Adapted from: Schweizer et al. 2006 [17])

Current protein designation	Current gene designation	New protein designation	New gene designation
Human epithelial keratin			
K1	*KRT1*	K1	*KRT1*
K2e	*KRT2A*	K2	*KRT2*
K3	*KRT3*	K3	*KRT3*
K4	*KRT4*	K4	*KRT4*
K5	*KRT5*	K5	*KRT5*
K6a	*KRT6A*	K6a	*KRT6A*
K6b	*KRT6B*	K6b	*KRT6B*
K6e/h	no designation	K6c	*KRT6C*
K7	*KRT7*	K7	*KRT7*
K8	*KRT8*	K8	*KRT8*
K6irs1	no designation	K71	*KRT71*
K6irs2	no designation	K72	*KRT72*
K6irs3	no designation	K73	*KRT73*
K6irs4	no designation	K74	*KRT74*
K6hf	no designation	K75	*KRT75*
K2p	*KRT2B*	K76	*KRT76*
K1b	no designation	K77	*KRT77*
K5b	no designation	K78	*KRT78*
K6l	no designation	K79	*KRT79*
Kb20	no designation	K80	*KRT80*
Human hair keratins			
Hb1, K2.9	*KRTHB1*	K81	*KRT81*
Hb2	*KRTHB2*	K82	*KRT82*
Hb3, K2.10	*KRTHB3*	K83	*KRT83*
Hb4	*KRTHB4*	K84	*KRT84*
Hb5, K2.12	*KRTHB5*	K85	*KRT85*
Hb6, K2.11	*KRTHB6*	K86	*KRT86*
Nonhuman epithelial/ hair keratins			
—	—	—	—
Human keratin pseudogenes			
	ψhHbD , Kb31P		*KRT121P*
	ψhHbC , Kb30P		*KRT122P*
	ψhHbB , Kb29P		*KRT123P*
	ψhHbA , Kb28P		*KRT124P*
	ψKRTH		*KRT125P*
	ψKRTG , Kb19P		*KRT126P*
	ψKRTF		*KRT127P*
	ψKRTE		*KRT128P*

5-1.15 Keratin Filament Structure

All intermediate filaments, of which keratins comprise the largest family, have the same tripartite domain structure. A central α-helical "rod" domain is flanked by poorly-structured 'head' and 'tail' regions at the N- and C- terminus, respectively (Figure 5-06). The size and amino acid sequence of the head and tail domains varies considerably amongst the intermediate filaments and confers each its specific properties and expression pattern. The central rod domain comprises four α-helical sub-domains interrupted by non-helical linker regions and makes up over 50% of the keratin protein which is involved in filament assembly. The α-helix occurs because of a heptad repeat of amino acid residues and has a specific arrangement of hydrophobic residues that forms a hydrophobic stripe running around the right-handed α-helix in a left-handed manner. This allows two helices to associate to produce the coiled-coil dimer building block of the intermediate filament (Figure 5-07).

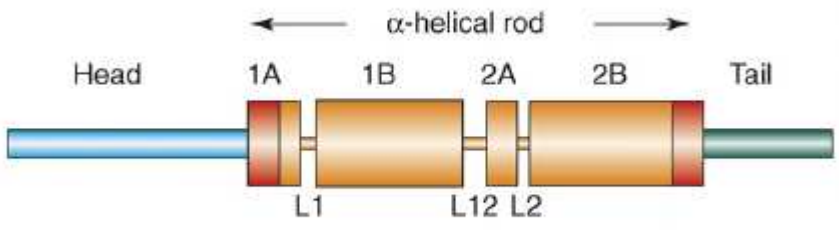


Figure 5-06: Schematic representation of intermediate filament protein structure showing the central α-helical rod domain flanked by non-helical "head" and "tail". In the rod domain a heptad repeat is interrupted at three conserved location by linker sequences L1, L12 and L2 producing subdomains 1A, 1B, 2A, 2B. The ends of the rod domain (shown in red) contain highly conserved 15-20 amino acid sequences. (Adapted from: Gu and Coulombe 2007 [18])

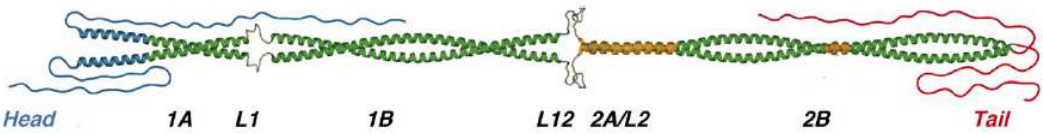


Figure 5-07: The dimer structure of an intermediate filament comprising a pair of parallel chains lying in axial register along the central rod domain. (Adapted from: Parry et al. 2007 [19])

In vivo keratins initially heterodimerise in the specific type I and type II pairs that are expressed in each cell. However, keratins have been described as promiscuous heterodimers [19] as more than one type I chain may be able to interact with the same type II chain and vice versa e.g. K5 with either K14 or K15 in the basal keratinocytes of stratified squamous epithelium. The dimers then undergo a rapid aggregation into tetramers in a half staggered anti-parallel arrangement with overlap of their 1B segments (Figure 5-08).

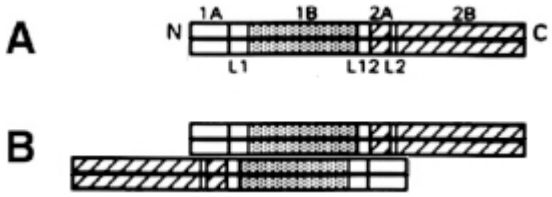


Figure 5-08: (A) Parallel arrangement of keratin type I and type II chains to form a heterodimer. (B) Assembly of keratin heterodimers in a staggered anti-parallel manner to form tetramers.

Tetramers assemble to form unit length filaments (ULFs) which elongate by reorganisation of their heterodimeric building blocks before annealing longitudinally to form immature intermediate filaments. A final radial compaction step occurs to pack the strands together more closely in the mature intermediate filament which is approximately 10nm in diameter and several μm long [19] (Figure

5-09). The mature keratin filaments assemble to form the cytoskeleton of the cell which needs to be a dynamic structure able to respond to external stimuli by disassembly and turnover. The keratin cytoskeleton has a highly dynamic composition *in vivo* because intermediate filaments are able to exchange subunits along their entire length unlike the other structural components, actin and microtubules, which only allow subunit exchange at their ends.

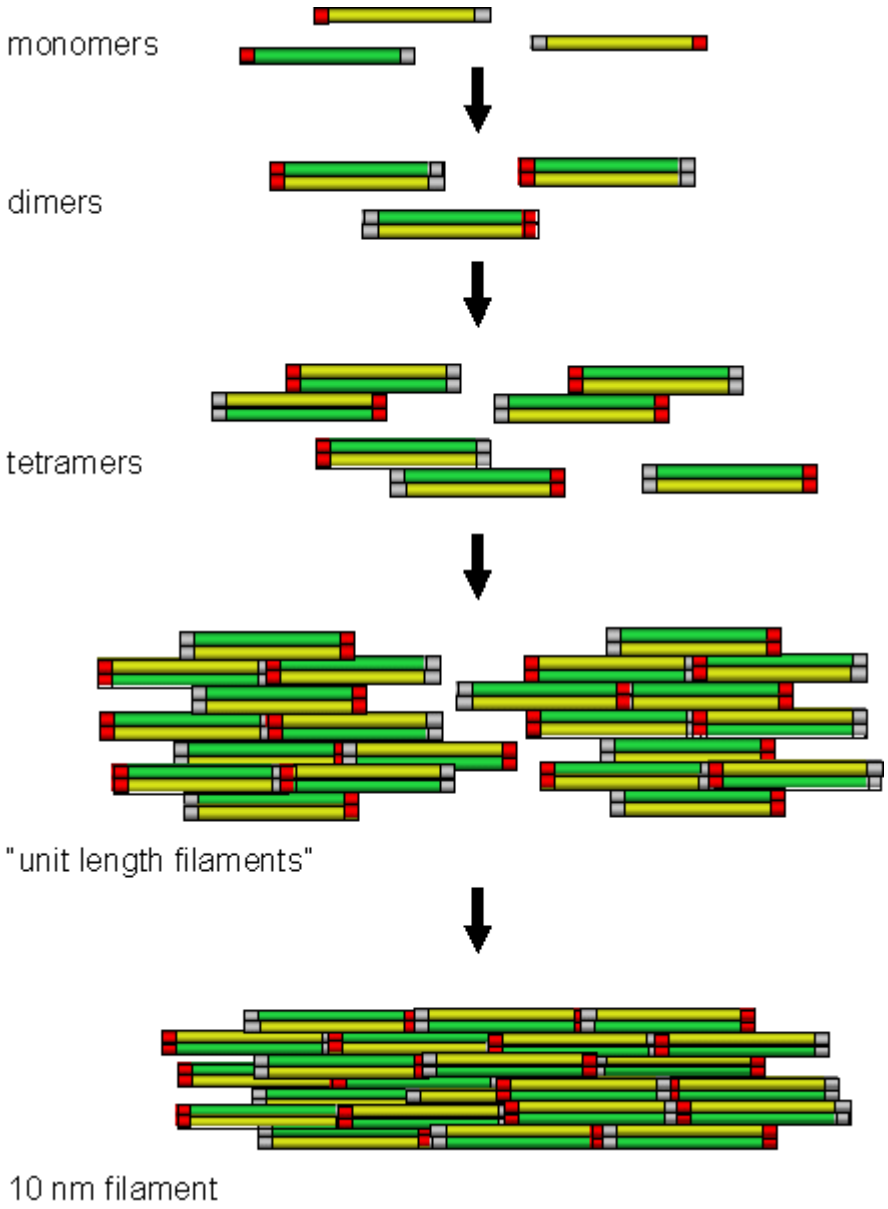


Figure 5-09: Schematic of the current understanding of keratin intermediate filament assembly. (From: www.interfil.org)

5-1.16 Keratin Expression

Keratinocytes express matched pairs of type I and type II keratin polypeptides in a tissue- and differentiation-specific manner [134]. For example, oral stratified squamous epithelium expresses keratins K5 and K14/K15 in the basal layer but not in the more differentiated suprabasal layers. Similarly, anatomical site specificity is shown by the keratinising epithelium of the gingivae expressing K1/10 in the suprabasal layers in addition to K4/13 and K6/16 which are also expressed in the non-keratinising buccal epithelium (Figure 5-10).

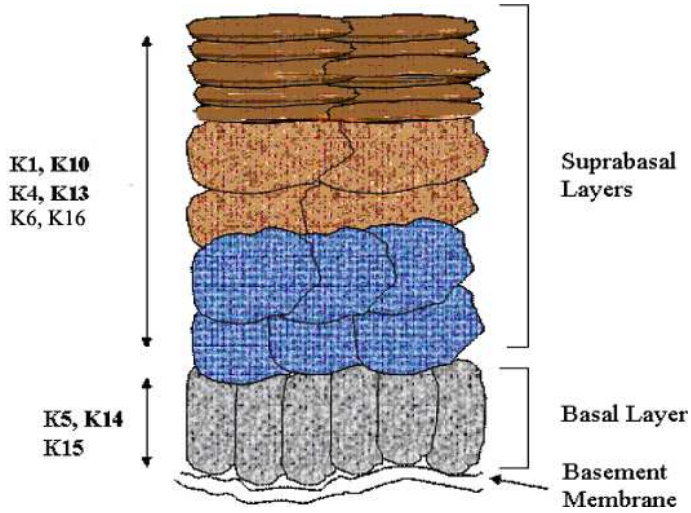


Figure 5-10: Schematic representation of differentiation specific keratin expression in normal oral epithelium.

5-1.17 Pathological Keratin Expression

Keratin gene expression is extremely sensitive to changes in cytokine signalling which results in alterations of the keratinocyte phenotype that can be detected by immunohistochemistry with anti-keratin antibodies. The activated keratinocyte phenotype of cutaneous wound healing is one example that presents as upregulation of K6/16 and K17 in the suprabasal keratinocytes with down-

regulation of K1/10 [136]. The mechanisms involved and roles of these hyperproliferation related keratins are only recently beginning to be understood and may include provision of a more flexible cytoskeletal framework for keratinocyte migration [137] as well as a direct control function in the healing process [138]. Altered keratinocyte phenotypes have also been reported in a wide array of benign and malignant oral epithelial diseases including lichen planus, hyperkeratosis, squamous cell carcinomas and odontogenic cysts [139, 140] indicating keratin gene expression is also responsive to pathological signalling.

Changes in keratin expression are widely used as detectable markers of underlying pathology by antibody binding in IHC. This is facilitated by keratins being immensely stable in comparison to other more volatile proteins that also respond to pathological stimulus e.g. the heat shock proteins that act as molecular chaperones in many protein-protein interactions necessary for protein structural conformation [141] and therefore potentially also antibody binding. Therefore, the traumatic tissue biopsy process itself is less likely to directly influence keratin protein expression observed by IHC, if samples are snap frozen or rapidly formalin fixed, implying that any change in keratin expression is representative of the underlying pathology and not of the sampling procedure.

5-1.18 Keratin Expression in OSF

With the epithelial abnormalities consistently observed in OSF and keratins being known to respond to pathological signalling in other oral PMDs, an altered keratin expression pattern in OSF may be detectable. There is some evidence of this in the literature. Vaidya et. al. (1998) [142] studied the expression of keratins in buccal

mucosa from seven OSF patients by immunoblotting. They reported loss of K5 in five of seven samples, and K14 downregulation in two samples whilst K8 was only expressed in three of the seven samples.

In 2006 the same group reported an IHC study utilising formalin fixed paraffin embedded tissue sections where the intensity of staining was assessed to determine differences in keratin expression [143]. In this study K5 expression was observed in 48 (96%) out of 50 OSF samples throughout the basal epithelium and abnormally the suprabasal epithelium. Its usual basal specific type I partner K14 was expressed in just one (2%) of 50 OSF samples and they did not report on K15, the other type 1 keratin, which is known to partner K5 when K14 is downregulated [144].

Ranganathan et. al. (2006) [143] also showed K8 staining (basal and suprabasal) in five (10%) of 50 OSF samples and as K8 is usually a simple epithelium specific keratin any expression in the stratified squamous epithelium of the oral mucosa is abnormal. Of the possible type 1 partners [145] only K18 was reported where basal specific staining was seen in five (12%) of 50 OSF samples.

Other specific keratin expression patterns reported by Ranganathan et. al. (2006) [143] were K4 in one (2%) of 50 OSF samples and again its usual partner K13 was not reported. K4/13 in the literature are reported as a differentiation specific keratins in non-keratinised oral epithelium [139] being supplanted by K1/10 in keratinised normal oral epithelium [146]. Ranganathan et. al. (2006) showed K1 expression in 16 (32%) of their OSF samples but did not report in K10 expression.

These aberrant results maybe related to the original site of biopsy in OSF patients being keratinised or non-keratinised mucosa but this detail is not reported in the study.

Keratin expression in normal epithelium has been well characterised in the literature [17, 133, 134, 146, 147] so normal tissue samples were used as controls by Ranganathan et. al. (2006) [143]. However, they observed significantly different staining patterns from that reported in the literature. Differentiation specific K4 expression was observed in nine (90%) of ten samples throughout the basal as well as the suprabasal epithelium. The other differentiation specific keratin studied was K1 and this was observed predominantly suprabasally in four (40%) of ten normal samples. The conventionally basal expression of K5/14, because they are known to be down-regulated in normal differentiation was also not observed in this study. K5 staining was reported as basal and suprabasal in all ten (100%) of normal samples and K14 was seen in just two (20%) of normal tissues but again throughout suprabasal as well as basal epithelium. As expected the simple epithelia specific K8 was not seen in any normal samples but its type 1 partner K18, also reported in the literature as simple epithelia specific in normal tissue, was observed throughout the basal and suprabasal epithelium of five (50%) of ten normal samples. To explain these abnormalities Ranganathan et. al. (2006) reported that their ‘normal’ tissue samples were taken from “patients who had come for tooth extraction and did not have good oral hygiene…therefore…it is possible these tissues were not fully normal”.

Taken together there is some evidence that OSF epithelium expresses keratins in an abnormal fashion and this would be best categorised by IHC of fresh frozen tissue specimens to eliminate the effect of antigen masking.

5-1.19 An Altered Keratinocyte Phenotype in OSF Epithelium?

In summary, we hypothesise that an altered keratinocyte phenotype occurs in OSF affected epithelium. Our aim was to determine the keratinocyte phenotype of OSF affected epithelium by the expression of keratins. The overall keratin expression profile can then be compared to the changes seen in other OMLs such as LP, dysplasia and hyperkeratosis and conserved changes may provide an insight into the pathogenesis and management of OSF. Characterisation of the keratinocyte phenotype in OSF may also provide specific immunohistological parameters for definitive diagnosis of the condition.

5-2.01 MATERIALS AND METHODS

5-2.02 Tissue Samples and Preparation

Samples of site-matched normal (6 patients) and OSF tissue (28 patients) were collected from a Sri Lankan population in the University of Peradeniya and the import of tissue samples was compliant with the Human Tissue Act 2004. Four normal samples and 22 OSF biopsies were obtained from normally non-keratinising buccal mucosa and the remainder from keratinised sites such as the tongue and gingivae (Table 5-04). OSF was diagnosed by clinical presentation and confirmed by histological evaluation of formalin fixed paraffin embedded tissue sections. OSF lesions were graded for severity using conventional histological parameters [16, 129] by Professor W. M. Tilakaratne, Centre for Clinical and Diagnostic Oral Sciences, Queen Mary School of Medicine and Dentistry, University of London. Biopsy specimens were snap frozen and stored in sealed containers at -80^{o}C until required. Tissue blocks were mounted on cork discs in OCT cryo-mounting medium (VWR, Pennsylvania, USA) and serial 6µm sections cut in a cryostat at -25^{o}C (Bright Instruments, Cambridge UK). Sections were thaw mounted on Superfrost + coated slides (Menzel-Glaser, Germany) and stored in sealed microscope slide boxes at -80^{o}C until required for staining.

Table 5-04: Tissue samples used in this study. (OSF samples were graded for severity by histological parameters and also for the presence of dysplasia which when present was mild dysplasia in all cases).

Sample	Biopsy Site	OSF Histological Grade			Dysplasia
		Early	Intermediate	Advanced	
OSF 1	Buccal			+	-
OSF 2	Tongue		+		+
OSF 3	Buccal		+		-
OSF 4	Buccal		+		-
OSF 5	Buccal			+	+
OSF 6	Buccal			+	-
OSF 7	Lip		+		+
OSF 8	Buccal		+		+
OSF 9	Lip		+		-
OSF 10	Buccal			+	+
OSF 11	Lip			+	-
OSF12	Buccal		+		-
OSF 21	Buccal		+		-
OSF 22	Buccal	+			-
OSF 23	Buccal	+			-
OSF 24	Buccal	+			-
OSF 25	Tongue		+		-
OSF 26	Buccal	+			-
OSF 27	Buccal		+		-
OSF 28	Buccal			+	-
OSF 29	Buccal		+		+
OSF 30	Buccal	+			-
OSF 32	Buccal		+		-
OSF 33	Buccal		+		+
OSF 35	Lip		+		-
OSF 41	Buccal		+		+
OSF 42	Buccal			+	-
OSF 61	Buccal	+			-

N0	Tongue
N1	Buccal
N2	Buccal
N6	Buccal
N13	Buccal
NA	Gingivae

5-2.03 Antibodies and Immunohistochemistry

Twenty-two different monoclonal mouse antibodies (mAbs) (Table 5-05) were obtained, either from commercial sources or raised in house by culturing the respective hybridomas in Dulbecco's modified Eagle's medium with 10% foetal calf serum and the supernatants from confluent cultures stored, refrigerated, in 0.2% (w/v) sodium azide. In house mAbs were gifts from Professor I. M. Leigh, Centre for Cutaneous Research, Queen Mary School of Medicine and Dentistry, University of London.

Table 5-05: Mouse monoclonal antibodies used in this study.

Clone	Specificity	Working Dilution	Supplier
LHK1	K1	1:500	in house
LHK2e	K2	1:1	in house
6B10	K4	1:10	Sigma-Aldrich, USA
LHK6B	K6	1:100	in house
LP5K	K7	1:1	in house
LE41	K8	1:1	in house
Ks9.20+Ks9.28	K9	1:10	Progen, Germany
DE-K10	K10	1:50	Neomarkers, USA
RKSE60	K10	1:10	Biogenesis, UK
LHP2	K10	1:1	in house
1C7	K13	1:1	in house
1C7+2D7	K13	1:10	Neomarkers, USA
LL001	K14	1:500	In house
LHK15	K15	1:500	In house
LL025	K16	1:500	In house
E3	K17	1:10	Dako, Denmark.
LP34	K6+K18	1:500	in house
LE61**	K18	1:100	in house
LE65**	K18	1:10	in house
CY-90	K18	1:10	Sigma-Aldrich, USA
LP2K	K19	1:100	in house
RCK108	K19	1:100	Dako, Denmark.

***Data using recombinant polypeptides has shown these antibodies bind complexes of K8/18 rather than individual polypeptides.* [148, 149]

Three layer indirect immunohistochemical staining was performed on frozen tissue sections utilising the DAKO LSAB+ kit (Dako, Cambridgeshire UK). Sections were first air dried for 20 minutes after removal from -80^{o}C storage and blocked with 5% normal goat serum (Vector Labs, USA) in PBS for 30 minutes at room temperature. This and subsequent incubation stages were carried out at room temperature in a humid chamber with utmost care taken to prevent the sections drying out. After washing in 3 changes of PBS for 5 minutes each the sections were incubated in primary antibody diluted in PBS for 1 hour before washing again (3 changes of PBS for 5 minutes each) and adding the biotinylated multilink secondary for 30 minutes. Another wash stage followed and the streptavidin complex added for 30 minutes. This was then washed away and colour developed using 3, 3'-diaminobenzidine (DAB) (Vector Labs, California USA). Counterstaining of cell nuclei was with Mayers haematoxylin (Sigma-Aldrich, USA)

and the tissues mounted in Vectamount (Vector Labs, USA) under glass coverslips. Where available immunohistochemical staining was performed with several different mAbs raised against the same keratin polypeptide, to determine reproducibility of the staining pattern.

5-2.04 Control of Variables

For this experimental design the dependant variable, in which we were interested, was keratin expression within the epithelium. Control of extraneous variables involved use of a standardised staining protocols. The monoclonal antibodies utilised were generally well characterised with either extensive usage within the Centre for Cutaneous Research and the Centre for Clinical and Diagnostic Oral Sciences of Queen Mary School of Medicine and Dentistry, University of London or commercially available and having been previously used in publications within peer reviewed journals. Staining protocols were optimised for each antibody either from departmental experience in their use or by repeat staining runs on normal tissues or cells which expressed the relevant keratin e.g. MCF7 cells for K8/18 and K19. In addition the keratin expression pattern of normal oral epithelium, has been published in a number of peer reviewed articles with, in general, consistent keratin expression patterns amongst studies utilising the same antibody clone on frozen tissue sections [133, 144, 147, 150, 151].

Each staining run included procedural controls such as the substitution of primary mAb for PBS and the staining of normal epithelial samples or cells with known keratin expression patterns.

5-2.05 Data Analysis

The staining data were analysed by visual assessment and quantified by image analysis. Slides were photographed on a Microphot-FXA microscope with Coolpix 990 camera (Nikon, Japan) and the images assembled using Adobe Photoshop CS2 v9.0.2 (Adobe Systems Inc., USA).

5-2.06 Visual Assessment

Each slide was visually assessed, initially, independently by 2 observers and agreement was reached by consensus where initial assessment differed. Representative sections of slides showing positive staining were graded on a three point equally weighted scale (+, ++ or +++) for staining in the basal layer and the suprabasal compartment of the epithelium at 150x magnification. Reference slides with (+) representing 0-33% stained surface area, (++) 34-66% and (+++) 67-100% stained surface area were provided. No attempt was made to quantify intensity of DAB staining.

A Cumulative Staining Quotient (CSQ) for OSF and normal samples was calculated using the formula:

$$\textit{Cumulative Staining Quotient (CSQ)} = \frac{\textit{number of (+) in all samples}}{\textit{number of samples}} \times 100$$

The CSQ therefore semi-quantifies the visual assessment grading and shows gross differences in staining pattern between the whole population of OSF samples and normal samples. Results were further subdivided into samples from normally keratinised or non-keratinised biopsy sites as OSF pathology is known to be associated with inappropriate hyper-keratinisation of the epithelium and

anatomically normal keratinisation may conceal any pathological effects on keratin expression.

5-2.07 Pixel Analysis

Due to the inherent subjectivity of the visual assessment approach a more quantitative approach by image analysis was also completed for each slide showing a different staining pattern in normal and OSF samples. Pixel analysis was performed on the photographed digital images, using a modification of the approach described by Elie (2003) [152]. Photographs were taken at 100x magnification with identical optical and digital zoom camera settings. In Photoshop the epithelium was carefully selected from the tissue section using the 'lasso tool' and the total pixel count in the selected area recorded from the Histogram palette (Figure 5-11). For keratinised samples the superficial keratin layer was not selected as part of the epithelium as it is often poorly retained in frozen sections and the dense keratinisation in these squames may obscure antibody binding sites [133]. A new image was created by cutting and pasting the selected epithelium and from this the basal and suprabasal compartments carefully delineated by the lasso tool to give the pixel count for each segment of the epithelium. To separate brown DAB staining from the blue haematoxylin counterstain a colour range selection was performed using the same sampled colour with RGB values of 160, 100 and 100 respectively as shown in the 'colour palette' of the software. The range of colours selected was set to 100 on the 'fuzziness slider' which selects other parts of the image by the degree their colour is related to the sample colour. To confirm that all DAB stained sections of the image were selected, these pixels were cut from the image to leave just the counterstained areas. Any DAB stained areas

remaining when the selected pixels were removed, for example with very dark brown staining, were manually selected and employed for the evaluation using the 'plus eyedropper' tool of the software. The DAB pixel count in the basal and suprabasal compartments of the epithelium was recorded and calculated as a percentage of the total basal or suprabasal pixel count (that will include DAB and haematoxylin). Quantification in this manner permitted use of the Mann Whitney U test to determine statistically significant differences in the DAB staining between normal and OSF samples.

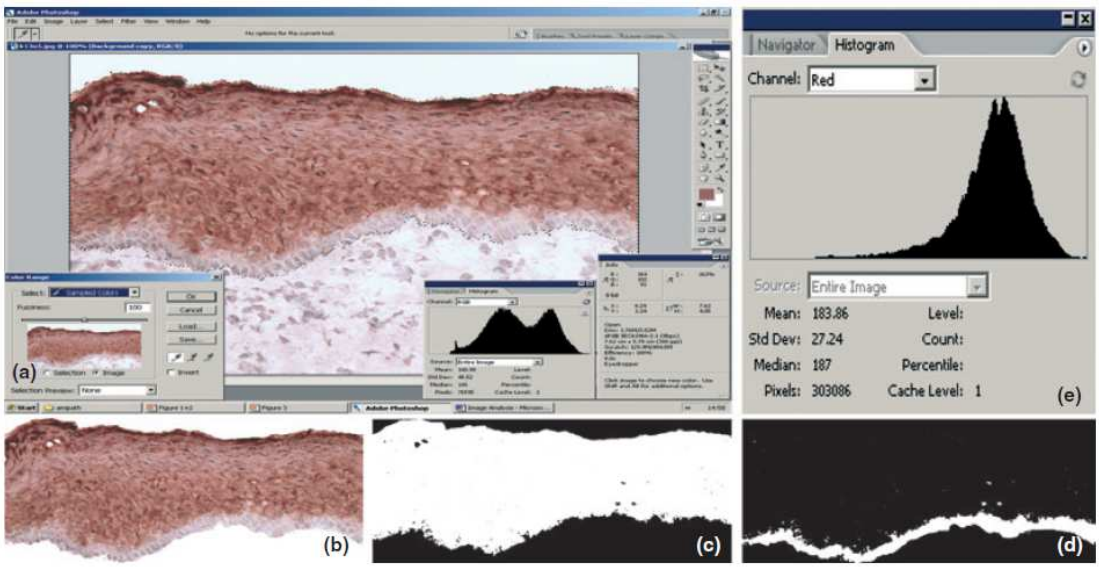


Figure 5-11: Quantitative analysis of DAB immunostained and haematoxylin counterstained tissue images. (a) Original images imported into Adobe Photoshop CS2 v9.0.2 (Adobe Systems Inc., USA), (b) with epithelium delineated and (c) DAB staining specifically selected or (d) haematoxylin counterstain selected by adjustment of 'colour selection' properties. (e) the pixel count of the selected area is given in the 'histogram palette'. (From: Lalli et. al. (2008) [20])

5-3.01 RESULTS

5-3.02 Suprabasal Keratins (K1/K10, K2, K4/K13, K6/16 and K9)

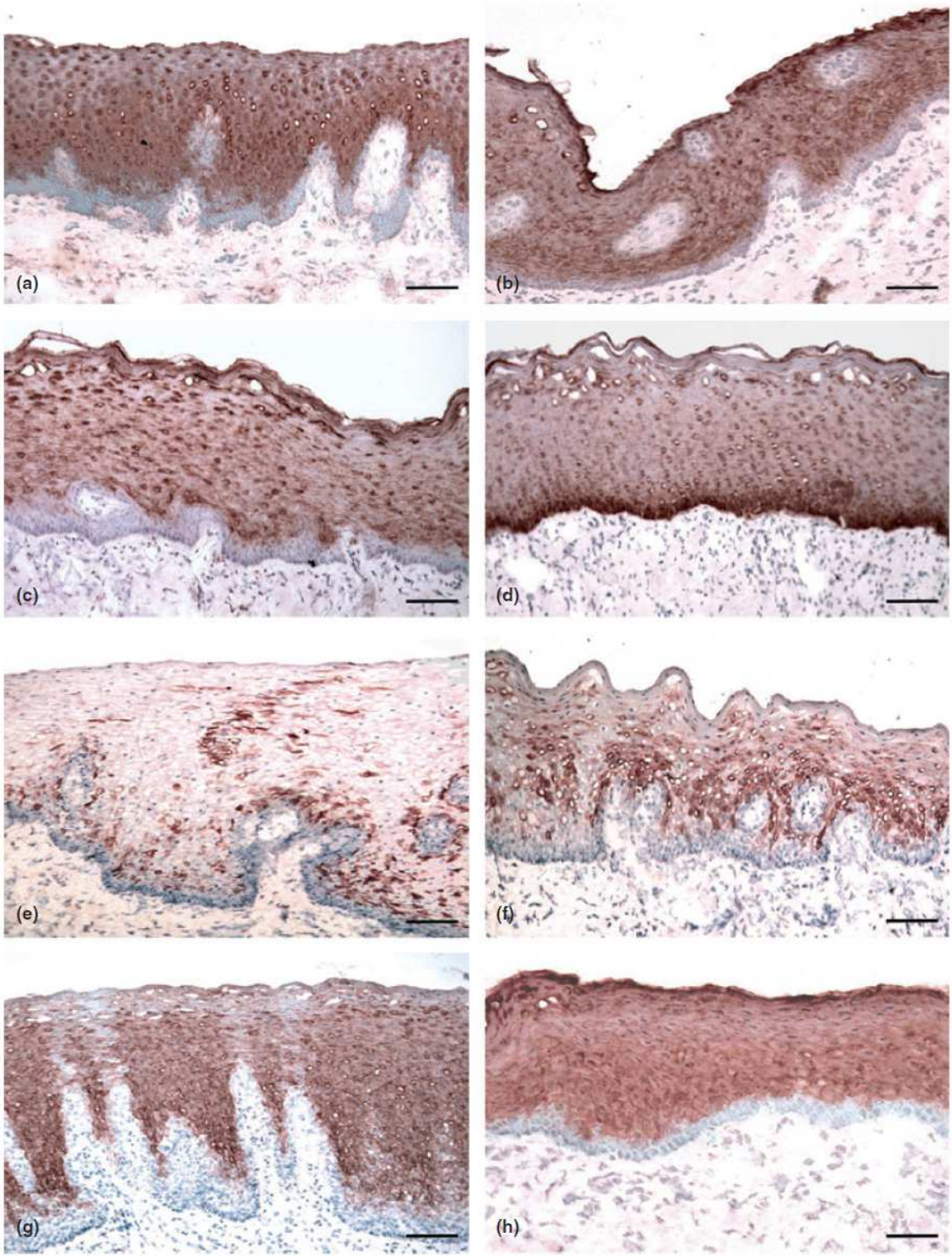


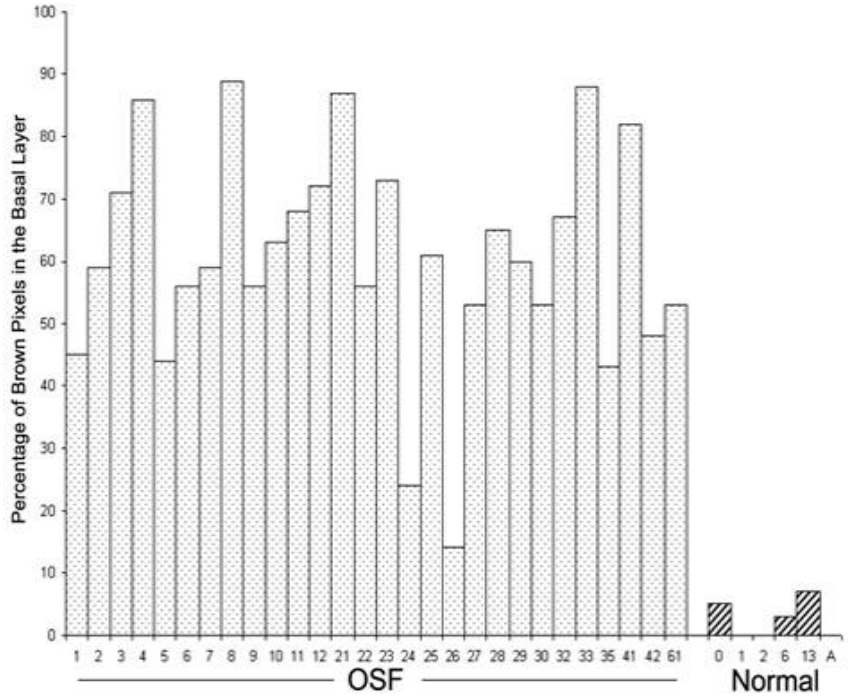
Figure 5-12: Immunohistochemical staining of normal oral (a, c, e and g) and OSF lesional (b, d, f, and h) epithelium for the suprabasal keratins. K16 (a and b), K6 (c and d), K1 (e and f) and K13 (g and h). Bar = 50μm.(Adapted from Lalli et. al. (2008) [20])

5-3.03 Keratins 6/16

K16 staining was entirely suprabasal with similar expression in all OSF and normal samples (Figure 5-12: images a and b) confirmed by a suprabasal CSQ of 92% and 94%, respectively (Table 5-06). K6 was also strongly suprabasal (CSQ 94%) in normal epithelia with no expression in the basal layer (CSQ 0%) (Figure 5-12: image c) whilst in OSF, K6 was detectable in both the basal (CSQ 70%) and suprabasal layers (CSQ 87%) (Figure 5-12: image d). The increase in basal K6 expression in OSF compared to normal was highly significant ($p<0.001$) whilst suprabasal K6 expression was unaffected by OSF ($p>0.05$) (Figure 5-13). In summary, it appears that K16 expression is unaffected by OSF pathology but its type II partner K6 is upregulated in basal keratinocytes.

Table 5-06: Cumulative Staining Quotient (CSQ) of immunostaining in normal and OSF samples. Results for biopsy samples from normally keratinised and non-keratinised samples are shown where a difference was observed.

Keratin	Cumulative Staining Quotient (%)			
	Basal		Suprabasal	
	Normal	OSF	Normal	OSF
K1 (all biopsy sites)	0	0	33	65
K1 (non-keratinised sites only)	0	0	17	65
K1 (keratinised sites only)	0	0	67	67
K10 (all biopsy sites)	0	0	33	69
K10 (non-keratinised sites only)	0	0	17	62
K10 (keratinised sites only)	0	0	67	67
K6	0	70	94	87
K16	0	0	94	92
K4	0	0	94	95
K13	0	0	94	96
K2	0	0	11	1
K14	100	100	100	96
K15	100	99	0	0
K17	61	58	17	44
K19 (all samples)	22	0	0	0
K19 (non keratinised sites only)	33	0	0	0
K19 (keratinised sites only)	0	0	0	0



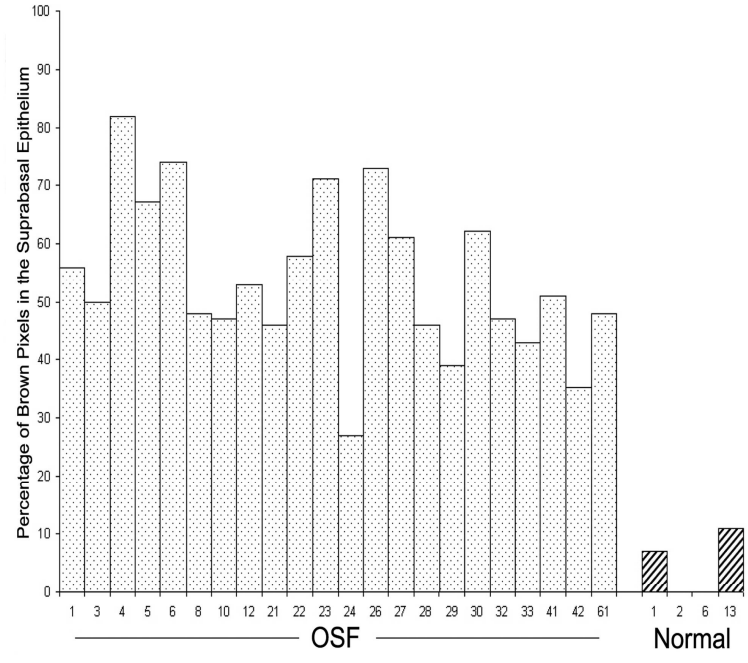
Samples	% DAB stained pixels	
	Basal	Suprabasal
OSF 1	45	97
OSF 2	59	100
OSF 3	71	90
OSF 4	86	92
OSF 5	44	90
OSF 6	56	93
OSF 7	59	88
OSF 8	89	82
OSF 9	56	90
OSF 10	63	97
OSF 11	68	96
OSF 12	72	99
OSF 21	87	98
OSF 22	56	67
OSF 23	73	90
OSF 24	24	55
OSF 25	61	84
OSF 26	14	57
OSF 27	53	71
OSF 28	65	37
OSF 29	60	83
OSF 30	53	68
OSF 32	67	60
OSF 33	88	97
OSF 35	43	61
OSF 41	82	95
OSF 42	48	67
OSF 61	53	89
N0	5	92
N1	0	89
N2	0	99
N6	3	94
N13	7	83
NA	0	85
U statistic	168.0	101.0
P value	1.49E-06	0.465938

Figure 5-13: Increased K6 expression in the basal layer of OSF epithelium compared to normal (p<0.001) whereas suprabasal K6 expression is unchanged.

5-3.04 Keratins 1/10

The expression of K1 and K10 was entirely suprabasal and comparable in all samples indicating this keratin pair is co-expressed in suprabasal keratinocytes. In normal epithelia K1/10 was expressed strongest in samples from keratinised biopsy sites with a CSQ of 67% for both keratins whilst the non-keratinised buccal epithelia displayed more limited staining represented by a CSQ of 17% (Figure 5-12 image e and Table 5-06). All OSF tissues, from both keratinised and non-keratinised biopsy sites, were strongly K1/10 positive with CSQs of 65% for K1 and 69% for K10 (Figure 5-12: image f and Table 5-06). There was no difference between OSF samples from keratinised and non-keratinised sites e.g. CSQs of 65% and 67% for K1 in non-keratinsed and keratinised samples, respectively (Table 5-06). For non-keratinised biopsy sites, increased K1/10 expression in OSF compared to normal was highly significant (p<0.001) whilst normally keratinised

sites appear qualitatively unaffected with a CSQ of 67% for both K1 and K10 although the number of relevant samples is too low for meaningful statistical analysis (Figure 5-14 and Table 5-06). Overall, it appears that the hyperkeratinisation associated K1/10 are upregulated in OSF to a level comparable with naturally keratinised oral epithelium.



Sample	% DAB stained pixels		
	All biopsy sites		Non-keratinised only
	Basal	Suprabasal	Suprabasal
OSF 1	0	56	56
OSF 2	0	43	-
OSF 3	0	50	50
OSF 4	0	82	82
OSF 5	0	67	67
OSF 6	0	74	74
OSF 7	0	42	-
OSF 8	0	48	48
OSF 9	0	19	-
OSF 10	0	47	47
OSF 11	0	61	-
OSF12	0	53	53
OSF 21	0	46	46
OSF 22	0	58	58
OSF 23	0	71	71
OSF 24	0	27	27
OSF 25	0	56	-
OSF 26	0	73	73
OSF 27	0	61	61
OSF 28	0	46	46
OSF 29	0	39	39
OSF 30	0	62	62
OSF 32	0	47	47
OSF 33	0	43	43
OSF 35	0	66	-
OSF 41	0	51	51
OSF 42	0	35	35
OSF 61	0	48	48
N0	0	49	-
N1	0	7	7
N2	0	0	0
N6	0	0	0
N13	0	11	11
NA	0	44	-
U statistic	84	148.0	88.0
P value	1	0.00225	0.000134

Figure 5-14: Increased suprabasal K1 expression in OSF affected oral epithelium originally from non-keratinised sites of the oral cavity, compared to normal epithelium.

5-3.05 Keratin 2

K2 was detected in a few upper suprabasal keratinocytes in the 2 out of 6 normal tissues, which originated from the keratinised surfaces of the tongue and gingivae with no expression in the normally non-keratinised buccal mucosa samples. One out of 28 OSF samples was weakly positive with similar expression pattern to that seen in the two normal oral keratinised samples. This OSF sample originated from the tongue and therefore none of the other five OSF samples from keratinised

biopsy sites were positive indicating a potential reduction in K2 expression although the number of relevant samples was limited (Table 5-06).

5-3.06 Keratins 4/13

All OSF and normal tissue samples stained strongly throughout the suprabasal epithelium for K4/13 with CSQs around 95% (Figure 5-12: image g and h and Table 5-06). OSF pathology therefore appears not to influence the expression of these suprabasal keratins.

5-3.07 Keratin 9

No expression was detected in normal or OSF affected oral epithelium which is consistent with K9 being a palmo-plantar epidermis specific keratin.

5-3.08 Basal Keratins (K14, K15, K17 and K19)

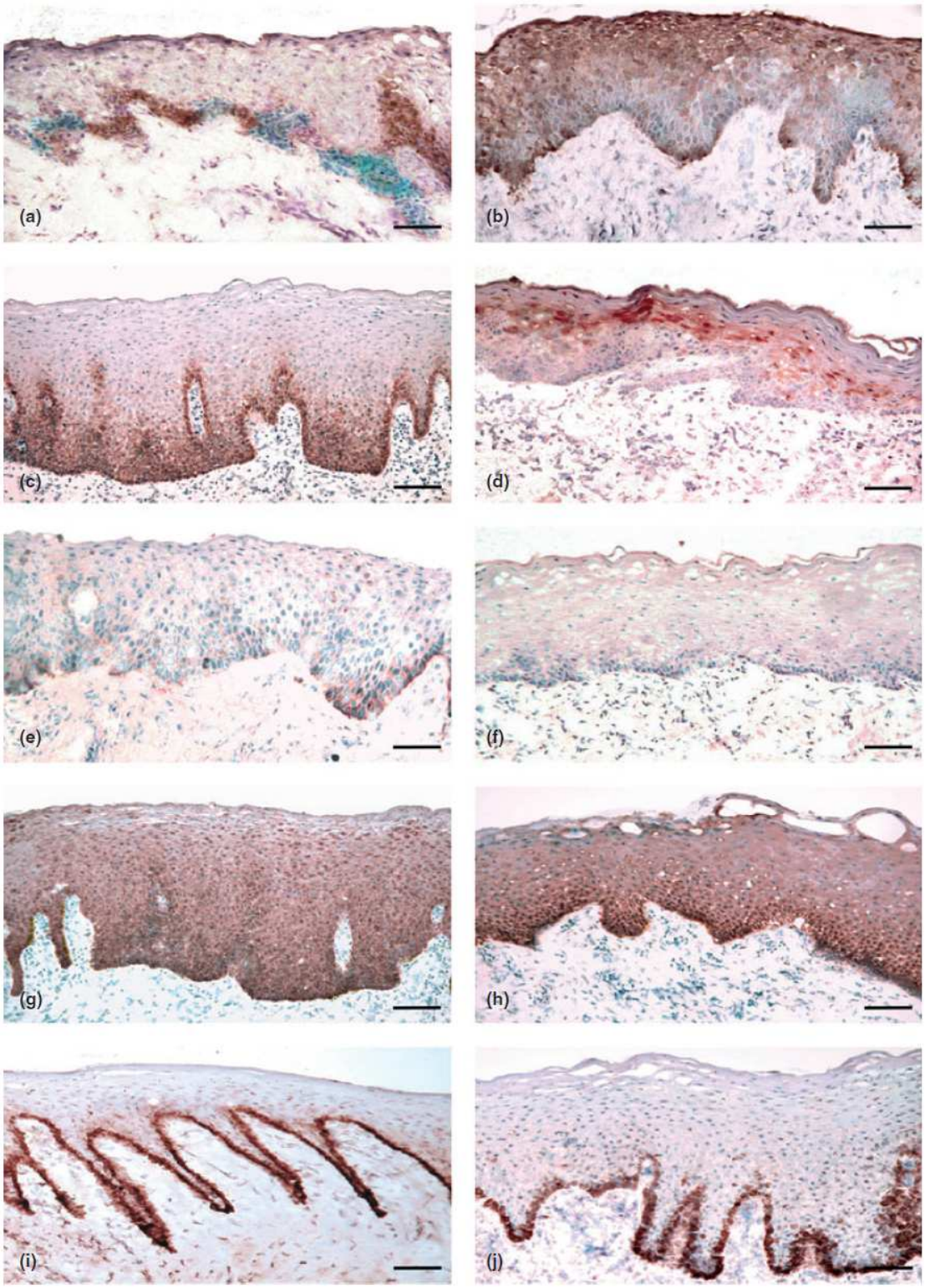


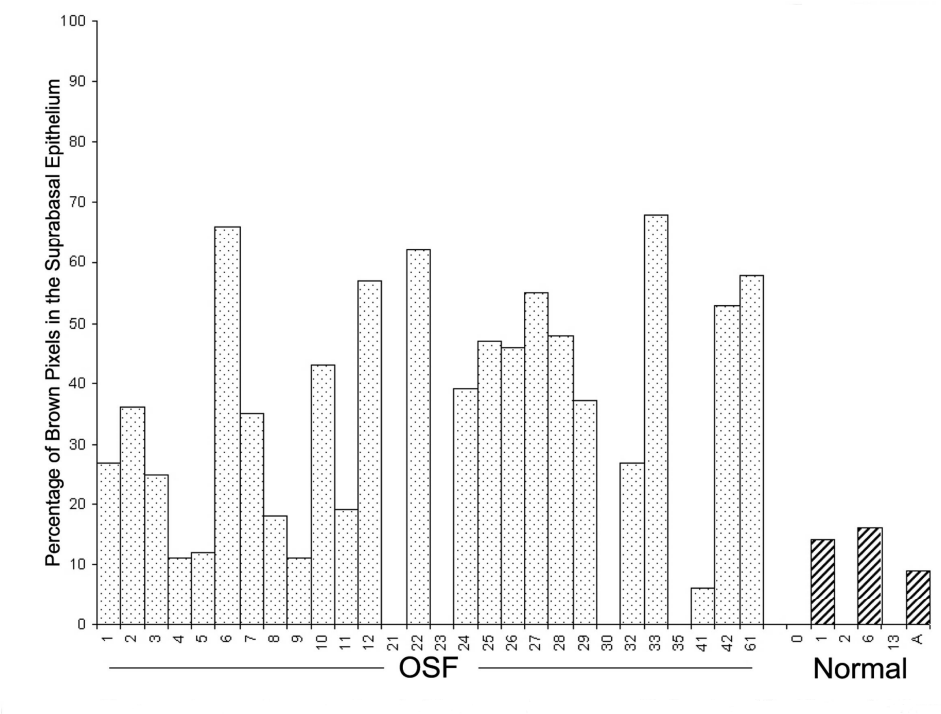
Figure 5-15: Immunohistochemical staining of normal oral (a, c, e, g and i) and OSF lesional (b, d, f, h and j) epithelium for the basal keratins. K17 (a and b showing isolated basal and suprabasal staining whilst c and d show both basal and suprabasal staining patterns), K19 (e and f), K14 (g and h) and K15 (i and j). Bar = 50μm. (Adapted from Lalli et. al. (2008) [20])

5-3.09 Keratin 17

K17 was detected in the basal layer of all normal samples but staining in suprabasal keratinocytes was only present in 50% of cases and generally confined to the lower suprabasal layers (Figure 5-15: images a and c). All OSF samples expressed K17 and although the staining varied considerably in intensity and distribution (Figure 5-15: images b and d) there was increased K17 in the suprabasal layers with CSQs of 17% in normal and 44% in OSF. Basal K17 expression appeared unaffected with CSQs of 61% in normal and 58% in OSF (Table 5-06).

5-3.10 Suprabasal K17 Expression Correlates with OSF Severity

Quantification by pixel analysis confirms this global picture of unchanged basal expression ($p>0.5$) and increased suprabasal expression ($p<0.01$) (Figure 5-16). This image analysis data correlated with histological severity shows there is no significant difference between normal levels of suprabasal expression and early OSF lesions ($p>0.05$) but with intermediate OSF there is a moderately significant increase ($p<0.05$) and there is a highly significant ($p<0.01$) increase in suprabasal K17 expression in the advanced OSF samples (Table 5-07 and Figure 5-17).



Sample	% DAB stained pixels	
	Basal	Suprabasal
OSF 1	0	27
OSF 2	22	36
OSF 3	28	25
OSF 4	44	11
OSF 5	36	12
OSF 6	81	66
OSF 7	56	35
OSF 8	47	18
OSF 9	34	11
OSF 10	41	43
OSF 11	2	19
OSF12	39	57
OSF 21	87	0
OSF 22	47	62
OSF 23	54	0
OSF 24	12	39
OSF 25	52	47
OSF 26	60	46
OSF 27	39	55
OSF 28	3	48
OSF 29	60	37
OSF 30	44	0
OSF 32	16	27
OSF 33	0	68
OSF 35	42	0
OSF 41	100	6
OSF 42	40	53
OSF 61	39	58
N0	14	0
N1	57	14
N2	11	0
N6	100	16
N13	45	0
NA	31	9
U statistic	85.5	141.0
P value	0.94739	0.00796

Figure 5-16: Increased suprabasal K17 expression in OSF epithelium compared to normal epithelium (p<0.01) whereas basal K17 expression is unchanged.

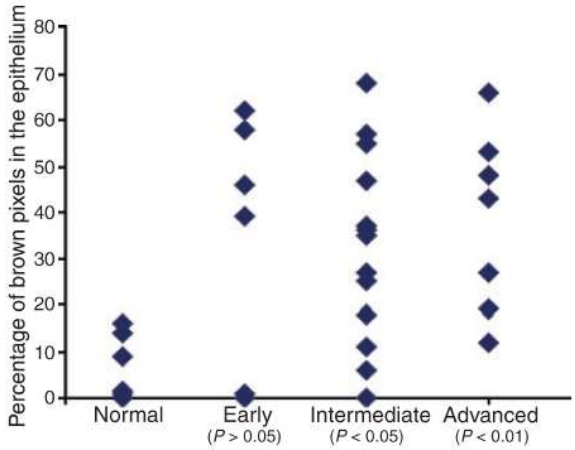


Figure 5-17: Suprabasal expression of K17 in OSF epithelium shown by image analysis of DAB immunostaining and correlated with histological grading of disease severity. No statistically significant difference is measurable between early lesions and normal epithelium but increased K17 is evident with intermediate pathology and advanced lesions display highly significant (p<0.01) K17 suprabasal increased expression.(From: Lalli et. al (2008) [20])

Table 5-07: Analysis by Mann Whitney U Test of normal against OSF populations for keratin expression and correlation with disease severity.

Sample	Brown (DAB) Coloured Pixels as a Percentage of All Pixels in The Epithelial Compartment							
	K17							
			EAR	INT	ADV	EAR	INT	ADV
	B	SB	B	B	B	SB	SB	SB
OSF 1	0	27	-	-	0	-	-	27
OSF 2	22	36	-	22	-	-	36	-
OSF 3	28	25	-	28	-	-	25	-
OSF 4	44	11	-	44	-	-	11	-
OSF 5	36	12	-	-	36	-	-	12
OSF 6	81	66	-	-	81	-	-	66
OSF 7	56	35	-	56	-	-	35	-
OSF 8	47	18	-	47	-	-	18	-
OSF 9	34	11	-	34	-	-	11	-
OSF 10	41	43	-	-	41	-	-	43
OSF 11	2	19	-	-	2	-	-	19
OSF12	39	57	-	39	-	-	57	-
OSF 21	87	0	-	87	-	-	0	-
OSF 22	47	62	47	-	-	62	-	-
OSF 23	54	0	54	-	-	0	-	-
OSF 24	12	39	12	-	-	39	-	-
OSF 25	52	47	-	52	-		47	-
OSF 26	60	46	60	-	-	46		-
OSF 27	39	55	-	39	-	-	55	-
OSF 28	3	48	-	-	3	-	-	48
OSF 29	60	37	-	60	-	-	37	-
OSF 30	44	0	44	-	-	0		-
OSF 32	16	27	-	16	-	-	27	-
OSF 33	0	68	-	0	-	-	68	-
OSF 35	42	0	-	42	-	-	0	-
OSF 41	100	6	-	100	-	-	6	-
OSF 42	40	53	-	-	40	-	-	53
OSF 61	39	58	39	-	-	58	-	-
N0	14	0	14	14	14	0	0	0
N1	57	14	57	57	57	14	14	14
N2	11	0	11	11	11	0	0	0
N6	100	16	100	100	100	16	16	16
N13	45	0	45	45	45	0	0	0
NA	31	9	31	31	31	9	9	9
U Statistic	85.5	141.0	20.0	48.5	28.0	27.0	74.0	40.0
P Value	0.947 P >0.05	0.00796 P <0.01	0.818 P >0.05	0.791 P >0.05	0.445 P >0.05	0.180 P >0.05	0.0233 P <0.05	0.00466 P <0.01

SB Suprabasal Epithelial Compartment
B Basal Epithelial Compartment
EAR OSF Histological Severity Grading - Early
INT OSF Histological Severity Grading - Intermediate
ADV OSF Histological Severity Grading – Advanced

5-3.11 Loss of Basal K17 Expression in OSF

The two extremes of the variable K17 expression pattern were assessed, that is those samples with entirely basal or suprabasal K17 expression, which showed that all samples with entirely basal staining were not advanced OSF lesions whilst all samples with complete loss of basal K17 staining were more advanced lesions (Table 5-08). Further indicating that suprabasal K17 expression in OSF maybe related to disease severity although loss of K17 basal expression only occurs in a

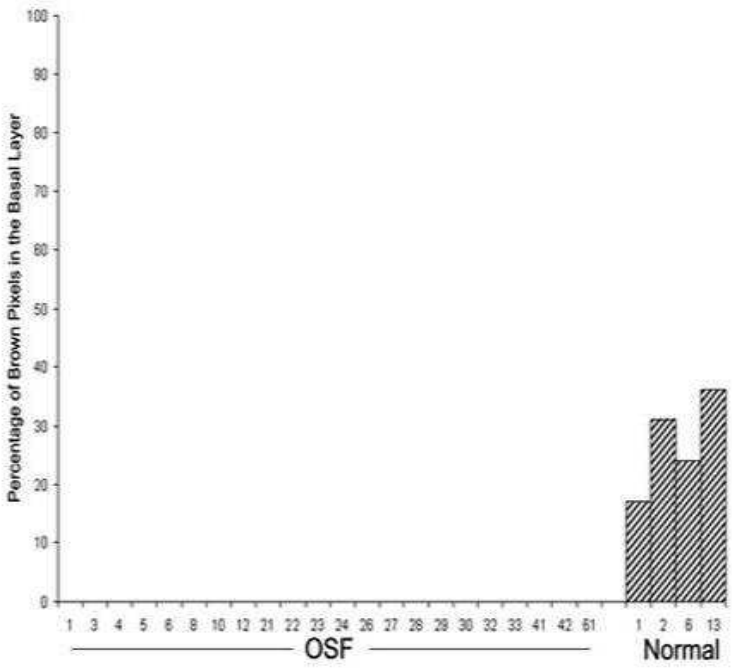
subset of these lesions. No other histological or clinical parameters, e.g. epithelial inflammation or atrophy, were assessed for correlation with K17 expression.

Table 5-08: Loss of basal K17 expression in a subset of the more advance OSF cases whilst no samples without suprabasal K17 were advanced lesions.

Sample	K17 Staining		OSF Histological Grading		
	Basal	Suprabasal	Early	Intermediate	Advanced
OSF 1	-	+			+
OSF 11	-	+			+
OSF 28	-	++			+
OSF 33*	-	++		+	
OSF 4	++	-		+	
OSF 21	+++	-		+	
OSF 23	++	-	+		
OSF 35	++	-		+	
OSF 41*	+++	-		+	
*Evidence of dysplastic change in the epithelium					

5-3.12 Keratin 19

K19 staining was discontinuous and limited to the basal layer of non-keratinised normal epithelia (Figure 5-15: image e) with no expression in normal keratinised epithelium. There was also no expression in any OSF samples whether from keratinised or non-keratinised sites (Figure 5-15: image f). The apparent down-regulation of K19 in OSF was confirmed by a highly significant reduction in staining in the basal layer of non-keratinised epithelia ($p<0.001$) compared with the normal controls (Figure 5-18).



Sample	% DAB Stained pixels	
	All biopsy sites	Non-keratinised only
	Basal	Basal
OSF 1	0	0
OSF 2	0	-
OSF 3	0	0
OSF 4	0	0
OSF 5	0	0
OSF 6	0	0
OSF 7	0	-
OSF 8	0	0
OSF 9	0	-
OSF 10	0	0
OSF 11	0	-
OSF12	0	0
OSF 21	0	0
OSF 22	0	0
OSF 23	0	0
OSF 24	0	0
OSF 25	0	-
OSF 26	0	0
OSF 27	0	0
OSF 28	0	0
OSF 29	0	0
OSF 30	0	0
OSF 32	0	0
OSF 33	0	0
OSF 35	0	-
OSF 41	0	0
OSF 42	0	0
OSF 61	0	0
N0	0	-
N1	17	17
N2	31	31
N6	24	24
N13	36	36
NA	0	-
U Statistic	140	88.0
P Value	0.009345	0.000134

Figure 5-18: Loss of K19 expression in OSF epithelium compared to non-keratinised normal epithelium ($p<0.001$).

5-3.13 Keratin 14/15

K14 staining was present homogenously throughout the basal and suprabasal layers (Figure 5-15: images g and h) whilst K15 was detected in the entire basal layer (Figure 5-15: images I and j) of both normal and OSF samples implying the disease did not influence expression of these two keratins.

5-3.14 Simple Epithelial Keratins (K7, K8 and K18)

No staining for K7, K8, K18 or the K8/18 complex was observed in normal or OSF samples. This is expected as oral epithelium is a stratified squamous tissue and these are simple epithelial keratins where differentiation related stimuli for keratin expression is not present.

5-3.15 Correlation between Visual Assessment and Pixel Analysis

There was a high level of correlation between the digital image pixel analysis and the visual assessment grading system shown by an overall correlation co-efficient value r = 0.90 ($p<0.001$). All non-staining keratins in basal or suprabasal compartments of the epithelium were disregarded (Table 5-09 and Figure 5-19). The heterogeneity of K17 staining resulted in the lowest correlation (r = 0.83) between pixel analysis and the more subjective visual grading. Conversely, the homogenous basal staining of K15 resulted in an almost perfect level of correlation (r= 0.99).

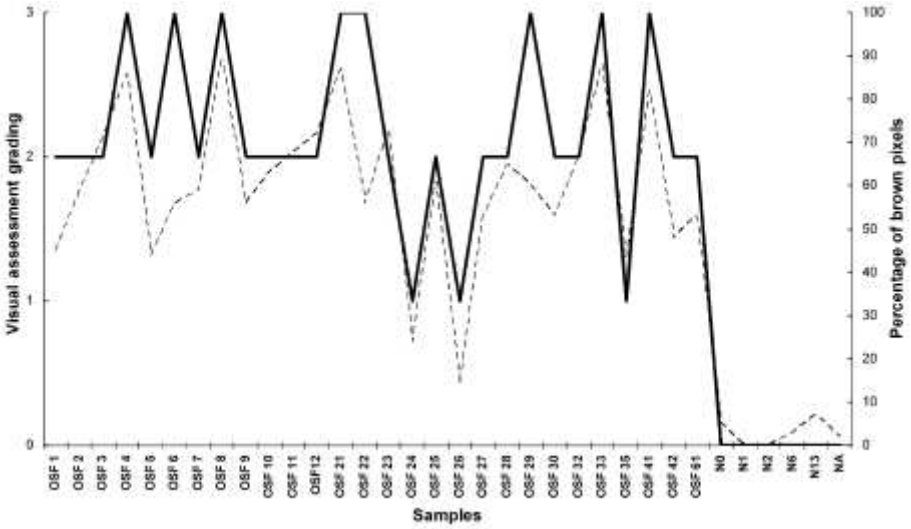


Figure 5-19: Correlation between visual assessment grading and image analysis of DAB Immunostaining for K6 in the basal layer of OSF and normal oral epithelium (r = 0.9). Dotted line represents image analysis and the solid line visual assessment.

Table 5-09: Keratin Immunostaining of normal and OSF epithelia showing the correlation between visual assessment of slides and pixel analysis of digital images.

Sample	DAB Staining in the Epithelium											
	K6		K1		K10		K15		K17		K19	
	B	SB	B	SB	B	SB	B	SB	B	SB	B	SB
OSF 1	++	+++	-	++	-	++	+++	-	-	+	-	-
	45	97	0	56	1	65	91	2	0	27	0	0
OSF 2	++	+++	-	++	-	++	++	-	++	++	-	-
	59	100	0	43	0	51	88	0	22	36	0	0
OSF 3	++	+++	-	++	-	++	+++	-	++	++	-	-
	71	90	0	50	0	43	97	0	28	25	0	0
OSF 4	+++	+++	-	+++	-	++	+++	-	++	-	-	-
	86	92	4	67	7	61	100	4	44	11	0	0
OSF 5	++	+++	-	++	-	++	+++	-	++	+	-	-
	44	90	0	82	0	49	98	0	36	12	0	0
OSF 6	+++	+++	-	++	-	++	+++	-	++	++	-	-
	56	93	0	74	0	60	96	0	81	66	0	0
OSF 7	++	+++	-	++	-	++	+++	-	++	++	-	-
	59	88	0	42	0	53	98	4	56	35	0	0
OSF 8	+++	+++	-	++	-	++	+++	-	++	++	-	-
	89	82	0	48	0	56	92	0	47	18	0	0
OSF 9	++	+++	-	+	-	+	+++	-	++	+	-	-
	56	90	0	19	0	13	100	8	34	11	0	0
OSF 10	++	+++	-	++	-	++	+++	-	++	++	-	-
	63	97	0	47	0	42	93	0	41	43	0	0
OSF 11	++	+++	-	++	-	++	+++	-	-	+	-	-
	68	96	0	61	0	53	99	1	2	19	0	0
OSF12	++	+++	-	++	-	++	+++	-	++	++	-	-
	72	99	1	53	8	82	91	0	39	57	0	0
OSF 21	+++	+++	-	++	-	+++	+++	-	+++	-	-	-
	87	98	0	46	0	59	97	6	87	0	0	0
OSF 22	++	+++	-	++	-	++	+++	-	++	++	-	-
	56	67	0	58	0	71	85	0	47	62	0	0
OSF 23	++	+++	-	++	-	+++	+++	-	++	-	-	-
	73	90	2	71	9	78	98	0	54	0	0	0
OSF 24	+	+++	-	+	-	++	+++	-	++	++	-	-
	24	55	0	27	0	44	100	2	12	39	0	0
OSF 25	++	+++	-	++	-	++	+++	-	++	++	-	-
	61	84	0	56	0	46	91	0	52	47	0	0
OSF 26	+	+++	-	++	-	++	+++	-	++	++	-	-
	14	57	4	73	0	62	93	0	60	46	0	0
OSF 27	++	+++	-	++	-	++	+++	-	++	++	-	-
	53	71	0	61	0	39	97	3	39	55	0	0
OSF 28	++	++	-	++	-	++	+++	-	-	++	-	-
	65	37	0	46	3	57	88	0	3	48	0	0
OSF 29	+++	+++	-	+	-	++	+++	-	++	++	-	-
	60	83	0	39	0	48	96	0	60	37	0	0
OSF 30	++	++	-	++	-	++	+++	-	++	-	-	-
	53	68	0	62	0	53	99	7	44	0	0	0
OSF 32	++	+++	-	++	-	++	+++	-	+	+	-	-
	67	60	0	47	0	41	98	0	16	27	0	0
OSF 33	+++	+++	-	++	-	++	+++	-	-	++	-	-
	88	97	0	43	0	59	92	0	0	68	0	0
OSF 35	+	++	-	++	-	++	+++	-	++	-	-	-
	43	61	0	66	0	67	98	0	42	0	0	0
OSF 41	+++	+++	-	++	-	++	+++	-	+++	-	-	-
	82	95	0	51	0	40	98	2	100	6	0	0
OSF 42	++	++	-	++	-	++	+++	-	+	++	-	-
	48	67	0	35	0	49	100	6	40	53	0	0
OSF 61	++	+++	-	++	-	++	+++	-	++	++	-	-
	53	89	0	48	0	55	93	0	39	58	0	0
N0	-	+++	-	++	-	++	+++	-	+	-	-	-
	5	92	0	49	0	31	92	0	14	0	0	0
N1	-	+++	-	+	-	+	+++	-	++	+	+	-
	0	89	0	7	0	16	98	0	57	14	17	0
N2	-	+++	-	-	-	-	+++	-	+	-	+	-
	0	99	0	0	0	0	99	0	11	0	31	0
N6	-	+++	-	-	-	-	+++	-	+++	+	+	-
	3	94	0	0	0	0	86	0	100	16	24	0
N13	-	+++	-	+	-	+	+++	-	++	-	+	-
	7	83	0	11	0	9	94	3	45	0	36	7
NA	-	++	-	++	-	++	+++	-	++	+	-	-
	0	85	0	44	0	53	100	5	31	9	0	0
Correlation	r = 0.90		r = 0.95		r = 0.96		r = 0.99		r = 0.83		r = 0.96	
P value	P<0.001		P<0.001		P<0.001		p<0.001		P<0.001		P<0.001	

B Basal Epithelial Compartment
SB Suprabasal Epithelial Compartment
- No DAB staining
+ <33% of Epithelium DAB Stained
++ 33-66% of Epithelium DAB Stained
+++ >66% of Epithelium DAB Stained
DAB Coloured Pixels as a Percentage of the Total Pixel Count in the Epithelial Compartment

5-4.01 DISCUSSION

Epithelial-mesenchymal interactions play vital roles in keratinocyte differentiation, proliferation, migration and invasion [153, 154]. Alterations in keratin expression as a result of changes in the mesenchyme have also been reported using both *in vitro* [155] and *in vivo* models [156]. In OSF an imbalance of collagen metabolism causes excessive deposition of collagen leading to abnormal mesenchyme, which could affect the overlying epithelium. Two known benign connective tissue disorders of the epidermis, hypertrophic and keloid scars, are also characterised by abnormal collagen metabolism with considerable similarities to OSF and it is known that the pathogenesis of these condiitons involves perturbed epithelial-mesenchymal interactions with abnormality of keratinocyte phenotype in the epidermis [133, 136, 157]. Therefore, we hypothesise that keratinocytes in OSF epithelium could be altered, which would implicate them in the disease pathogenesis, as well as their predisposition to oral cancer.

5-4.02 Hyperkeratinisation of OSF Epithelium

In OSF, compared to non-keratinised normal epithelium, there is increased suprabasal expression of K1/10 (Figure 5-12: image c) which is a feature of oral hyperkeratotic lesions [133]. Keratinised normal oral epithelium also shows similar expression of K1/10 indicating this to be a specific molecular marker of keratinisation [139]. The histologically apparent keratosis in many OSF lesions is therefore consistent with the increase in K1/10 expression. Increased K1/10 expression has also been associated with K2 upregulation in oral dysplastic lesions which is distinct from the keratinisation seen in oral lichen planus (LP). In LP related keratosis, K1/10 upregulation occurs independently of K2 induction [133].

OSF lesions thus mirror mirrors LP in that K1/10 expression is upregulated without a concomitant increase in K2 despite the presence of dysplasia. LP is an autoimmune disorder therefore the similarities in K1/10 and K2 regulation seen in OSF may indicate an immunological pathogenesis although it is believed that keratinisation in OSF is due to direct mechanical trauma from the coarse fibres of the areca nut. LP is histologically characterised by a dense sub-epithelial band of lymphocytes, which produces a massive inflammatory infiltrate with resultant high levels of pro-inflammatory cytokines that induce abnormal keratinocyte differentiation. Whilst inflammatory cells are not overtly evident in OSF lesions the dense fibrosis and reduced vasculature of OSF sub-epithelial tissues may allow for long term build up of inflammatory cytokines which can then affect the epithelium in a similar manner. This should also provide a mechanism for malignant change as persistent tissue inflammation is implicated in carcinogenesis [158].

5-4.03 Specific Induction of K6 in the Basal Keratinocytes

In OSF we observed increased K6 expression in the basal layer of the epithelium but no change in the constitutive basal keratins K14 and K15 (Figure 5-12: image b and Figure 5-15: image g to j). The influence of OSF pathology on K5 expression could not be determined as a well characterised mAb, suitable for frozen sections, was not available. Commercially available anti-K5 antibody XM26 (Novocastra, Newcastle UK) has been used on formalin-fixed sections but with frozen tissue this mAb did not react. This confirms that it may not be possible to compare frozen and formalin-fixed samples for keratin staining using the same mAb [159, 160]. The only previous immunohistological study of keratin expression in OSF used formalin-fixed samples [143] and this may explain some of the inconsistencies in observed

expression patterns such as suprabasal expression of the basal specific K5 and lack of K14 expression in 80% of samples. Alternatively, this abnormal staining could be a feature of the mAbs employed and it is unclear whether site-matched normal and OSF tissues were used in the study. In addition, Ranganathan et. al. (2006) [143] did not use antibodies against K6, K17 and K19, the keratins found to be the most influenced by OSF pathology.

Induction of K6 expression in the basal layer of OSF epithelium creates a situation whereby a type II keratin is switched on without the expression of its normal type I partner K16. In non-keratinised epithelium heterogenous basal expression of K6 but not K16 has been reported previously [151] and only in the rete-ridges, using anti-K6 KA12 antibody. Unfortunately, no image of the staining was published so it is impossible to critically evaluate the reactivity. None of our normal oral tissues expressed K6 in the basal layer which is consistent with the literature using LHK6B [147] but the pattern of K6 expression in other PMDs is unknown. Interestingly, staining observed with LP34 was entirely suprabasal in both OSF and normal tissue and this antibody is commercially marketed for the detection of K6 and K18 (Cancer Research Technology, London. UK) or K5, K6 and K18 (Acris, Hiddenhausen, Germany), suggesting its precise reactivity is yet to be established. We have shown with multiple mAbs (LE61, LE65 and CY-90) that K8/K18 complex or K18 alone are not detectable in these samples, implying LP34 staining may be K6 specific. However, as K6 is present in the basal layer of OSF epithelium (Figure 5-12: image 1d), the lack of LP34 reactivity suggests that this mAb may not recognise K6 either. If LP34 was reacting with K5 then staining would be observed in the basal layer, but this was absent in normal samples indicating that

LP34 may also not detect K5. The LP34 staining in normal and OSF epithelium could be from cross-reactivity with another, as yet unidentified suprabasal protein, in a manner similar to anti-K14 LL001 antibody which stains both basal and suprabasal keratinocytes in normal buccal epithelium (Figure 5-15: image g), although K14 mRNA expression is restricted to the basal layer [144]. Alternatively, it is conceivable that the LP34 binding site on K6 may undergo a conformational change in K6/16 complexes to unmask the epitope. This does not occur in the basal layer of OSF epithelium as K16 is absent, whereas in the suprabasal layers both K6 and K16 are expressed, inducing LP34 binding. The conformation-specific-epitope phenomenon has been reported for a number of keratin antibodies [148, 149, 161-163] and requires further investigation as LP34 is widely used in diagnostic pathology. Nevertheless, LHK6B staining in the basal layer of oral epithelium appears to be a characteristic feature of OSF, in this study. Furthermore, as the basal layer predominantly contains proliferating cells and the suprabasal cells are derived from basal keratinocytes, anomalous expression of K6 in the basal layer is likely to influence subsequent differentiation related cellular features such as keratinisation and epithelial atrophy. The literature also reveals this K6 expression pattern is also exhibited by normal oral keratinocytes induced to differentiate in an organotypical culture [147] although the significance is unclear.

5-4.04 Reduced Expression of K19 and K17 in the Basal Keratinocytes

The sporadic K19 expression in the basal layer of normal nonkeratinised oral epithelia is consistent with that reported in the literature. Decreased K19 expression in OSF may be symptomatic of the keratinised state of the tissue as

K19 expression is known to be incompatible with keratinisation in normal oral epithelia [150, 164].

K17 has been described as both an oral epithelial basal cell marker [165] and a suprabasal hyperproliferation marker similar to K16 in epidermal wound healing [151, 166]. Our findings are consistent with the former as expression of K17 is predominantly in the basal layer of normal epithelium with sparse suprabasal expression in 50% of samples. Table 5-08 shows 4 out of 28 OSF samples in which there was no basal expression of K17 and 3 of these were amongst the most advanced OSF cases, whilst the other was classified as intermediate with evidence of dysplasia. Conversely, 5 out of 28 samples expressed basal but no suprabasal K17 and none were advanced cases. Loss of basal K17 appears to be a feature of certain advanced OSF pathologies in a particular cytokine environment such as the low level of interferon γ found in some OSF lesions [48]. This premise is consistent with the fact that interferon γ is known to induce K17 in keratinocytes [167, 168]. Perhaps K17 expression is more sensitive to interferon γ than K15 because K15 is also induced by interferon γ [169] but appears to be uninfluenced by OSF pathology (Figure 5-15: image I and j). Finally and despite the small number of samples, 4 out of 28 OSF tissues (14%) showing loss of K17 in the basal layer, as well as being biopsied from some of the most advanced lesions, is approximately the same number that would be projected to progress to OSCC although no direct correlation can be made to this effect.

5-4.05 Induction of K17 in the Suprabasal Layers of OSF Epithelium Correlates with Disease Severity

Although K17 expression in the basal layer was absent in a sub-set of the most severe cases, overall there was no correlation determinable between the basal K17 expression and disease severity. However, expression in the suprabasal layers correlated with histological grading of OSF severity whereby the most significant K17 increase appeared to occur in the most advanced cases (Figure 5-17). None of the other keratins influenced by OSF pathology showed any significant correlation with disease severity. These observations suggest that perturbation in K17 expression is perhaps a primary outcome of OSF pathology and the expression of other keratins is influenced by factors in addition to the disease pathology such as keratinisation or dysplasia. K17 expression has been linked in the literature with the development of OSCC in other *in vivo* immunohistochemical studies [140, 170] as well as *in vitro* molecular model systems [171]. However, the clinical significance of this is unknown as there is no clear link between histological severity of OSF and its clinical presentation or malignant change [16, 129].

5-4.06 Keratinisation in OSF has a Molecular Signature Distinct from that in Dysplasia

OMLs with dysplasia are often keratinised, however, hyperkeratinisation does not always lead to dysplasia. Virtually all of our OSF samples showed evidence of keratinisation but dysplastic changes were present only in seven samples. There are several lines of evidence to suggest that OSF keratinisation may be different from that seen in hyperkeratotic lesions or dysplasia. K16 has been reported to be

induced in some forms of dysplastic lesion [172] whilst we did not observe any change in the expression of this protein in OSF dysplastic epithelium. The complete absence of K19 in OSF epithelium observed in this study is in contrast to its reported induction in dysplasia [150]. Furthermore, in hyperkeratotic lesions and dysplasia there is a reported upregulation of keratin K2 [133] whilst in OSF hyperkeratotic or dysplastic epithelium K2 is not expression. Taken together these observations strongly suggest that keratinisation in OSF may have a molecular signature distinct from that observed in other hyperkeratotic lesions and dysplasia.

5-4.07 Validity of Visual Assessment for Keratin Immunostaining

There was a uniformly high level of correlation between pixel analysis and visual assessment of keratin staining in normal and OSF tissue (Table 5-09). This suggests the quicker and simpler but more subjective visual grading process is sufficiently accurate to obviate the need for the considerably more laborious but objective image analysis technique in quantifying keratin staining. However, it was noted that the most varied staining patterns observed in our study were with K17 and this resulted in the lowest level of correlation ($r = 0.83$) indicating that for heterogeneous keratin staining the more objective approach maybe valuable.

5-5.01 CONCLUSION

OSF pathology results in an altered keratinocyte phenotype presenting as an induction of keratinisation-specific keratins K1 and K10 in the suprabasal layers of OSF epithelium. In the basal layer there was increased K6 and decreased K19 expression. K17 expression in the basal layer was downregulated only in a sub-set of the most severe cases whereas in the suprabasal layers K17 expression increased with disease severity. This altered keratinocyte phenotype in OSF, suggests a potential mechanism for the chronic connective tissue pathology via perturbed epithelial-mesenchymal interactions and the specificity of changes may provide immunohistological diagnostic criteria for OSF although the specific clinical significance of these changes remains to be elucidated.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSIONS

Chapter 6

General Discussion and Conclusions

6-1.01 GENERAL DISCUSSION

The development of a screening protocol to target high-risk individuals for OSCC in the Tower Hamlets and Newham South Asian populations required it to be is both feasible within existing healthcare services and acceptable to the predominantly South Asian ethnic population involved.

6-1.02 The Value of a Community Advisory Group (CAG)

Specific behavioural and socio-economic factors affecting the South Asian population in the UK, such as tobacco and paan usage and financial deprivation, suggest they are at higher risk of OSCC than other groups within the UK. Targeting these characteristics in a culturally acceptable manner is feasible by the involvement of all relevant stakeholders in a community advisory group. The outcomes from the CAG need to be evaluated against the available data to prevent competing interests interfering with the primary objective of screening high-risk individuals. In our study the CAG wished to organise screening activity to enable equitable distribution of screening locations within the population whereas the most effective approach was to site screening activity in areas of highest footfall of high-risk individuals such as worship or shopping facilities.

In total 1596 individuals were screened between 2006 and 2008 in Tower Hamlets and Newham of which 86 were screened positive and referred to the local hospital

for definitive diagnosis. Of these 86 individuals, 21 (24%) failed to attend the secondary referral centre despite all possible efforts by the screening team and hospital service to encourage attendance. Reasons for non-attendance were investigated in patients who initially refused to comply with referral but subsequently did attend. Causes for initial non-attendance included language barriers, non-receipt of appointment letters and difficulty attending the hospital. This indicates that the model of screening, on a mobile dental unit in a community setting with culturally-matched advocates was able to overcome some specific barriers to access that still exist in the hospital setting for these high-risk individuals.

6-1.03 UK High-Risk South Asian Screening Outcomes compared to the Asian Subcontinent

Evaluation of our screening outcomes suggests that this screening protocol targeting high-risk individuals in a UK based South Asian population is more comparable to screening trials reported on the Asian subcontinent than other UK screening programmes. Most importantly this is observed in the issue of referral compliance where most UK based screening programmes report compliance rates approaching 100% contrasting to compliance rates of about 50% on the Indian subcontinent [62]. The Trivandrum Oral Cancer Screening Study [5] is the only trial reported as sufficiently robust for inclusion in a Cochrane review [70] and indicates a referral compliance rate of 63%. Our studies referral compliance rate of 76%, despite the measures taken to overcome barriers to healthcare service uptake, suggests this maybe an inherent property of the South Asian population studied.

Another similarity between our outcomes and those reported in the Trivandrum study is the proportion of screen positive individuals at 5.9% in Kerala and 86 (5.4%) out of a total of 1596 screened in Tower Hamlets and Newham. The reported screen positive rates for other UK based screening trials are 0.2% [173], 1.2% [174], 2.7% [175] and 5.5% [176] although study methodologies and populations are not comparable.

6-1.04 The Prevalence of OMLs in the High-Risk South Asian Population

Comparison of the prevalence of OMLs in South Asian populations suffers noticeably from the heterogeneity of methodology and reporting in the literature. The prevalence observed in our study at 54% of screened individuals is considerably higher than the 40% previously reported in the Tower Hamlets population by Pearson et. al. (2001) [23], although they were not attempting to target high-risk individuals. Reports from the Indian subcontinent suggest OML prevalence of 2-8% [24, 25, 122], again without targeting high-risk individuals. However the reporting of OMLs appears highly inconsistent as smoking related changes are the commonest lesions in one Indian population attending a hospital dental outpatients department of which 15% smoke [24] but not reported at all in another despite 9% of the screened population smoking [25]. Therefore, comparison with these prevalence figures is undertaken with caution although the high OML prevalence we report (54%) is similar to the 63% observed when targeting high-risk Indian groups such as mine-workers [123].

6-1.05 Gold Standard Screening Outcomes

Despite 1596 individual screenings in a high-risk population for oral cancer there were no OSCCs detected. Therefore, it is impossible to assess the sensitivity and specificity of screening on the basis of the ideal gold standard for one-off oral cancer screening programme, which is histologically confirmed OSCC. In reality this gold standard outcome is ethically impossible because it necessitates the biopsy of normal tissue to confirm those screened negative are actually disease free and determining a histological diagnosis solely for those screened positive risks introducing a verification bias. The fact that 24% of screen positive individuals referred to the hospital, did not attend for further investigation of their suspicious OMLs makes assessment of any gold standard outcome even less accurate. Therefore, a more appropriate gold standard outcome is proposed for UK based screening programmes taking into account the available healthcare services and its ethical obligations, the patient's risk factors and their OML diagnosis, which is also the specialist clinician's normal decision making process. This 'soft' gold standard is the outcome of whether a screen positive individual is discharged following definitive diagnosis or requires long term follow-up. On this basis our screening programme specificity was 98.7% with a PPV of 79% with 'sensitivity' and 'NPV' at 100% as would be expected from any definition of a gold standard that only assesses the positive screened individuals. For patient safety we assume the worst case scenario that all patients who did not comply with referral would have been reviewed and not discharged. These figures are comparable to the literature on OSCC screening where overall specificity is 97% and PPV 70% despite the heterogeneity of study methodologies reported [62]. The 79% PPV indicates that approximately 1 in 5 screen positive individuals were false

positives attributable to specific difficulties in assessing the screened population, such as differentiating discrete lesions from generalised paan-related staining which is consistent with the high prevalence of OMLs in this population. Low PPV is also unavoidable for a rare condition such as OSCC. Importantly, high specificity indicates very few false negatives amongst the discharged (healthy) population suggesting few patients who actually require follow-up would be discharged.

6-1.06 OMLs, Risk Factors and Screening Sensitivity

Qualitative evidence from the screening dentists suggested the presence of OMLs hampered their ability to make accurate diagnosis, in particular the presence of paan associated staining of the oral mucosa. The high prevalence of OMLs was confirmed by surveying the screened population during their consultation without adversely interfering with the primary objective of screening for OSCC. It was discovered that 46% of these lesions could not readily be diagnosed as benign conditions of no concern for malignancy. These complex lesions included the conventional PMDs [15] but also paan related staining of the oral mucosa and were predominantly at the high risk sites in the oral cavity (‘gutter area’ and buccal sulcus) for OSCC in a population with risk factors including smoking and areca nut usage. These complex OMLs, especially when more than one was present, were responsible for the high levels of false positives in screening referrals. Additionally, there was a direct link between the use of paan or tobacco and the number of OMLs detected whilst increasing age compounded the effect of these agents but did not itself result in increasing numbers of OMLs.

6-1.07 The Need for Diagnostic Aids in Oral Cancer Screening

From our evaluation of the screening protocol and outcomes two major issues needed resolving before a viable targeted screening programme could be contemplated. Firstly the issue of non-attendance of screen positive individuals at referral resulting in an inability to accurately evaluate screening outcomes and the potentially more important, but completely unquantifiable, effect on the screened individual. The second major issue is the high prevalence of OMLs in this population and in particular the complex lesions that cause difficulty in determining which lesions require further investigation for malignancy. These two issues are clearly inter-related as the difficulty in diagnosis leads to more false positive outcomes from screening activity with larger numbers of screen positive individuals referred and subsequently failing to attend for definitive diagnosis and treatment as required.

The standard process for definitive diagnosis of OMLs is a combination of gross (macroscopic) examination followed by histologic (microscopic) examination of a tissue section which may be aided by evaluation of the molecular properties of the lesion by immunohistochemistry of the tissue section. A viable intra-operative pathology consultation approach would be relatively straightforward on a mobile dental unit which could house facilities to immediately biopsy and snap freeze samples that would be couriered to a pathology laboratory for sectioning, staining and diagnosis. The screen positive patient could therefore have their definitive diagnosis within a few hours and potentially not need to attend the hospital.

PMDs are more problematic as although histology is valuable for diagnosis it is their potential to become OSCC that is the most important concern. Our screening outcomes suggest some PMDs, such as LP, can be readily diagnosed by direct visual examination and this would be enough to indicate the patient with relevant risk factors cannot be discharged from further follow-up. Leukoplakia presents a more difficult diagnostic decision for the screening dentist, especially with the paan staining commonly found in high-risk South Asian populations such that the majority of lesions referred as leukoplakia were actually benign hyperkeratosis. The presence of dysplastic change is the most widely used and reliable indicator of malignant potential in leukoplakia [33] and this can be assessed by intraoperative pathology support on fresh frozen sections. For the soft gold standard screening outcome the presence or absence of dysplasia would be appropriate for a positive result. From our experience an average of 2-3 individuals were screened positive on each screening day and would require intra-operative pathology support for definitive diagnosis which may be feasible within existing healthcare services.

6-1.08 OSF and Screening for Oral Cancer

In relation to South Asian populations, of particular concern is OSF because of the significant potential for malignant conversion (7-26%) [39] as well as the clinical complications associated with the fibrosis itself. OSF presents a specific problem in screening for OSCC because of its direct association with areca nut compounds [41] found in paan. However, OSF only occurs in a small proportion of areca compound users, such as the 7 out of 568 (1.2%), reported in our sample, suggesting that despite the clear association with areca nut the specific aetiology is complex and not clearly understood. Paan staining itself was a significant issue

reported by the dentists when attempting direct visual assessment of the oral mucosa during the screening programme. Therefore, histological methods to aid diagnosis of OSF are required to improve the sensitivity of OSCC screening in high-risk South Asian populations.

6-1.09 An Altered keratinocyte Phenotype in OSF

Keratin gene expression is extremely sensitive to pathological changes in cellular signalling which results in alterations of the keratinocyte phenotype that can be detected by IHC with anti-keratin antibodies. IHC evaluation of the epithelium in OSF indicates there are changes in the keratin expression pattern related to the OSF pathological process, suggesting that OSF is not a purely connective tissue disorder. Our results indicate OSF pathology results in an altered keratinocyte phenotype presenting as an induction of keratinisation-specific keratins K1 and K10 in the suprabasal layers of OSF epithelium. In the basal layer there was increased K6 and decreased K19 expression. K17 expression in the basal layer was downregulated only in a sub-set of the most severe cases whereas in the suprabasal layers K17 expression appeared to increase with disease severity. This altered keratinocyte phenotype in OSF, suggests a potential mechanism for the chronic connective tissue pathology via perturbed epithelial-mesenchymal interactions and the specificity of changes may provide immunohistological diagnostic criteria for OSF although the specific clinical significance of these changes remains unclear.

6-1.10 Limitations of this Study

The biggest regret I have is that 21 people who were screened at one of the oral cancer screening sessions were told they require further investigation and I can only assume they must have taken that to mean they might have OSCC. These 21 have never been definitively diagnosed and subsequently cleared or treated. The effects on an individual of this knowledge are unquantifiable and in the UK, in the 21st century, this is must never be allowed to happen in a cancer screening campaign. We have proposed a viable method of preventing this outcome by utilising intraoperative pathology support. Additionally, we can take some comfort from the much larger proportion of individuals who attended with an OML and were able to be reassured it was not OSCC. This direct positive effect is again essentially unquantifiable.

Data collection in the screening component of the study was a source of difficulty, specifically how much to collect. Other epidemiological studies, in which I have been involved and where a comprehensive data collection process is required, take tens of minutes to complete a questionnaire in addition to the oral examination. An example would be the Adult Dental Health Survey completed every 10 years and published by the Office for National Statistics. This approach would be unsuitable for a screening programme where high flow-through of patients is necessary and therefore data collection was limited to that which would normally be collected to assess OSCC risk. The disadvantage is difficulty in interpretation of findings such as the issues we encountered with descriptions of tobacco use as distinct from smoking. On the other hand our data set was complete which is often not the case when large amounts of information are

collected either due to errors by the data collectors or patients being unwilling or unable to give so much detailed information. This necessitates the use of statistical methods to quantify the errors and as each test requires its own set of assumptions this can introduce further errors to the data set which may not be so identifiable or quantifiable.

For the IHC component of the study we utilised a sample of Sri Lankan OSF and normal tissue samples which may not be directly comparable to UK populations. Not enough is known about OSF pathology to ensure that the molecular process is identical to that in the UK although the clinical presentation is indistinguishable. The Sri Lankan samples were utilised to permit a large enough study to demonstrate and evaluate any alterations in keratin expression especially with the need to further subdivide the samples by histological grading and the presence of dysplasia. In addition, the study published by Ranganathan et. al. (2006) [143] suggested that their abnormal keratin expression findings in apparently 'normal' samples were down to the collection process because the tissue was taken from patients attending for tooth extraction such that these samples maybe inflamed and although non-OSF not entirely 'normal'. Inflammation can be a pathological process and is known to result in changes in molecular markers such as the keratins [166]. Our Sri Lankan association permitted normal tissue to be sampled from non-inflamed buccal, tongue and gingivae with suitable ethical approval as was required at the time of harvesting. Also in the interests of homogeneity of sample handling and storage the whole population of analysed samples were collected from the same clinical facility in Peradeniya. This could be an important cause of heterogeneity especially when assessing molecular markers which are

responsive to wound healing processes as well as disease pathology. For example, with the facilities available at the Centre for Clinical and Diagnostic Oral Sciences we can snap freeze tissue in liquid nitrogen (-196^{o}C) and short-term store tissue in -80^{o}C freezers with long-term storage in liquid nitrogen, and transport samples either on dry ice (-78^{o}C) or liquid nitrogen depending on journey duration. Our Sri Lankan partners do not have access to such facilities and imported dry ice specifically for this study with the use of domestic freezers (-20^{o}C) for storage.

6-1.11 Scope for Further Work

The most important outcome of this study is whether a further screening programme in the Tower Hamlets and Newham population can be justified. The burden of oral cancer on the healthcare system remains an enigma for dentists who profess to specialise in diseases of the oral cavity because the most serious of these diseases should be easily managed, as most oral cancers occur with known risk factors and are visually detectable at an early stage. Yet the majority of oral cancers are still diagnosed at advanced stages when treatment is either ineffective or massively debilitating. The moral argument is therefore clearly that dentistry as a profession must eliminate the burden of oral cancer but the question of whether this can this be justified within the restricted resource environment of the healthcare system remains to be answered. Our study provides a model for effectively targeting a high-risk South Asian population conventionally believed to be poor users of screening services. Although financial considerations were not the primary objective of this study the cost per screening was approximately £24 in comparison to £45.50 for the NHS breast screening programme. This indicates

there may be scope to implement the necessary intra-operative pathology support to be a truly effective and ethical screening programme.

Our model of targeting high-risk populations based on their specific risk factors and utilising a mobile dental unit to deliver the screening service may provide a platform for further study. This approach could be targeted at other disadvantaged minority high risk groups who do not utilise conventional dental services. Alternatively, the ability to target a population with a high prevalence of OMLs from a mobile dental unit maybe useful in the evaluation of the new technology screening aids such as brush biopsy, vital staining or the light based systems. The major criticism of these systems appears to be their inability to improve sensitivity and specificity, over direct visual examination, when assessing abnormal but not clearly malignant lesions [13] i.e. the complex lesions detected during screening. Our screening approach would provide the ideal target population to optimise and evaluate these diagnostic aids.

The IHC analysis of keratins in fresh frozen OSF samples revealed some evidence of changes in keratin expression that may provide pathways for further investigation. The study of keratin protein expression and the expression profile of K17 in particular may provide markers of disease progression which need to be correlated with the clinical presentation and behaviour of the disease. The altered keratin expression of K6 in OSF may also facilitate the use of brush biopsy cytology samples where the tissue architecture is not available for examination. Co-staining for basal specific K15 and K6 may be able to determine which of the brush biopsy sample are basal cells (K15 positive) and which samples are

suprabasal OSF (K15 and K6 positive). The clinical value of this application is yet to be elucidated but markers of disease pathology may lead to improved diagnostic ability of these clinically important oral mucosa pathologies.

6-2.01 CONCLUSIONS

Oral cancer appears to be an ideal target for a screening programme because most oral cancers develop in visible lesions of the oral mucosa and most occur in response to known environmental carcinogens. Therefore unlike the majority of other cancers, OSCC can be detected early when treatment is simple and effective. A screening protocol to target high-risk individuals for OSCC in the Tower Hamlets and Newham South Asian populations is both feasible within existing healthcare services and culturally acceptable to the population involved. This approach is potentially so effective in overcoming barriers to access that positive screened patients then cannot utilise existing healthcare services for the follow-up treatment they need. In addition, the high-risk population presents with a high prevalence of visible oral mucosal lesions in which cancer can develop such that it renders screening by direct visual examination ineffective with a large proportion of false positive screening outcomes. An intra-operative pathology support service is required so immediate biopsy can be undertaken and a definitive diagnosis given without delay. This could be extended with immunohistochemical analysis of the fresh frozen sample to aid the diagnosis of oral mucosal lesions. Conditions such as paan associated Oral Submucous Fibrosis present particular diagnostic and management issues in this population and have characteristic keratin expression patterns as a result of the underlying pathology, which may be useful diagnostic markers.

Oral cancer screening can be effectively targeted at high-risk South Asian populations residing in the UK but ethical and effective screening programmes

should utilise intra-operative pathology support services to improve the diagnostic ability of the screening clinician.

REFERENCES

References

[1] Muwonge R, Ramadas K, Sankila R, Thara S, Thomas G, Vinoda J, et al. Role of tobacco smoking, chewing and alcohol drinking in the risk of oral cancer in Trivandrum, India: a nested case-control design using incident cancer cases. Oral Oncol 2008 May;44(5):446-54.

[2] Ramadas K, Arrossi S, Thara S, Thomas G, Jissa V, Fayette JM, et al. Which socio-demographic factors are associated with participation in oral cancer screening in the developing world? Results from a population-based screening project in India. Cancer Detect Prev 2008;32(2):109-15.

[3] Ramadas K, Sankaranarayanan R, Jacob BJ, Thomas G, Somanathan T, Mahe C, et al. Interim results from a cluster randomized controlled oral cancer screening trial in Kerala, India. Oral Oncol 2003 Sep;39(6):580-8.

[4] Sankaranarayanan R, Mathew B, Jacob BJ, Thomas G, Somanathan T, Pisani P, et al. Early findings from a community-based, cluster-randomized, controlled oral cancer screening trial in Kerala, India. The Trivandrum Oral Cancer Screening Study Group. Cancer 2000 Feb 1;88(3):664-73.

[5] Sankaranarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B, et al. Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. Lancet 2005 Jun 4-10;365(9475):1927-33.

[6] Subramanian S, Sankaranarayanan R, Bapat B, Somanathan T, Thomas G, Mathew B, et al. Cost-effectiveness of oral cancer screening: results from a cluster randomized controlled trial in India. Bull World Health Organ 2009 Mar;87(3):200-6.

[7] Avon SL. Oral mucosal lesions associated with use of quid. J Can Dent Assoc 2004 Apr;70(4):244-8.

[8] Cawson RA, Odell EW. Cawson's Essentials of Oral Pathology and Oral Medicine. 7th ed: Churchill Livingstone: 2002.

[9] Reichart PA, Philipsen HP. [Oral submucous fibrosis in a 31-year-old Indian women: first case report from Germany]. Mund Kiefer Gesichtschir 2006 May;10(3):192-6.

[10] NICE. Referral guidelines for suspected cancer. London: National Institute of Health and Clinical Excellence; 2005.

[11] NCIN. Cancer Incidence and Survival by Major Ethnic Group, England. 2002-2006: National Cancer Intelligence Network 2009.

[12] NCIN. Cancer Incidence by Deprivation, England 1995-2004: National Cancer Intelligence Network 2008.

[13] Lingen MW, Kalmar JR, Karrison T, Speight PM. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral Oncol 2008 Jan;44(1):10-22.

[14] Norton SA. Betel: consumption and consequences. J Am Acad Dermatol 1998 Jan;38(1):81-8.

[15] Warnakulasuriya S, Johnson NW, van der Waal I. Nomenclature and classification of potentially malignant disorders of the oral mucosa. J Oral Pathol Med 2007 Nov;36(10):575-80.

[16] Utsunomiya H, Tilakaratne WM, Oshiro K, Maruyama S, Suzuki M, Ida-Yonemochi H, et al. Extracellular matrix remodeling in oral submucous fibrosis: its stage-specific modes revealed by immunohistochemistry and in situ hybridization. J Oral Pathol Med 2005 Sep;34(8):498-507.

[17] Schweizer J, Bowden PE, Coulombe PA, Langbein L, Lane EB, Magin TM, et al. New consensus nomenclature for mammalian keratins. J Cell Biol 2006 Jul 17;174(2):169-74.
[18] Gu LH, Coulombe PA. Keratin function in skin epithelia: a broadening palette with surprising shades. Curr Opin Cell Biol 2007 Feb;19(1):13-23.
[19] Parry DA, Strelkov SV, Burkhard P, Aebi U, Herrmann H. Towards a molecular description of intermediate filament structure and assembly. Exp Cell Res 2007 Jun 10;313(10):2204-16.
[20] Lalli A, Tilakaratne WM, Ariyawardana A, Fitchett C, Leigh IM, Hagi-Pavli E, et al. An altered keratinocyte phenotype in oral submucous fibrosis: correlation of keratin K17 expression with disease severity. J Oral Pathol Med 2008 Apr;37(4):211-20.
[21] Barnes L, Eveson JW, Reichart P, Sidransky D. Pathology and Genetics of Head and Neck Tumours. Lyon: IARC Press: 2006.
[22] Eccles M, Mason J. How to develop cost-conscious guidelines. Health Technol Assess 2001;5(16):1-69.
[23] Pearson N, Croucher R, Marcenes W, O'Farrell M. Prevalence of oral lesions among a sample of Bangladeshi medical users aged 40 years and over living in Tower Hamlets, UK. Int Dent J 2001 Feb;51(1):30-4.
[24] Saraswathi TR, Ranganathan K, Shanmugam S, Sowmya R, Narasimhan PD, Gunaseelan R. Prevalence of oral lesions in relation to habits: Cross-sectional study in South India. Indian J Dent Res 2006 Jul-Sep;17(3):121-5.
[25] Byakodi R, Shipurkar A, Byakodi S, Marathe K. Prevalence of oral soft tissue lesions in Sangli, India. J Community Health 2011 Oct;36(5):756-9.
[26] Kinzler KWV, Bert The genetic basis of human cancer 2nd ed. New York: McGraw-Hill: 2002.
[27] Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011 Mar-Apr;61(2):69-90.
[28] WHO. International Statistical Classification of Diseases and Related Health Problems (10th Revision): World Health Organisation: 2010.
[29] Napier SS, Speight PM. Natural history of potentially malignant oral lesions and conditions: an overview of the literature. J Oral Pathol Med 2008 Jan;37(1):1-10.
[30] van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol 2009 Apr-May;45(4-5):317-23.
[31] Downer MC, Petti S. Leukoplakia prevalence estimate lower than expected. Evid Based Dent 2005;6(1):12; author reply 3-4.
[32] Scully C, Porter S. ABC of oral health. Oral cancer. BMJ 2000 Jul 8;321(7253):97-100.
[33] Schepman KP, van der Meij EH, Smeele LE, van der Waal I. Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. Oral Oncol 1998 Jul;34(4):270-5.
[34] Barnes L. Surgical pathology of the head and neck. 2nd edition. New York: Marcel Decker: 2001.
[35] Lodi G, Sardella A, Bez C, Demarosi F, Carrassi A. Interventions for treating oral leukoplakia. Cochrane Database Syst Rev 2006(4):CD001829.
[36] Holmstrup P. Can we prevent malignancy by treating premalignant lesions? Oral Oncol 2009 Jul;45(7):549-50.
[37] Kujan O, Oliver RJ, Khattab A, Roberts SA, Thakker N, Sloan P. Evaluation of a new binary system of grading oral epithelial dysplasia for prediction of malignant transformation. Oral Oncol 2006 Nov;42(10):987-93.

[38] Cox SC, Walker DM. Oral submucous fibrosis. A review. Aust Dent J 1996 Oct;41(5):294-9.
[39] Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. Oral Oncol 2006 Jul;42(6):561-8.
[40] Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. Oral Oncol 2009 Apr-May;45(4-5):309-16.
[41] IARC. Betel-quid and Areca-nut Chewing and Some Areca-nut-derived Nitrosamines: International Agency for Research on Cancer; 2004.
[42] Kerr AR. Efficacy of oral lycopene in the management of oral submucous fibrosis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007 Feb;103(2):214-5.
[43] Gupta SC, Khanna S, Singh M, Singh PA. Histological changes to palatal and paratubal muscles in oral submucous fibrosis. J Laryngol Otol 2000 Dec;114(12):947-50.
[44] Pindborg JJ. Oral submucous fibrosis: a review. Ann Acad Med Singapore 1989 Sep;18(5):603-7.
[45] Khanna JN, Andrade NN. Oral submucous fibrosis: a new concept in surgical management. Report of 100 cases. Int J Oral Maxillofac Surg 1995 Dec;24(6):433-9.
[46] Kakar PK, Puri RK, Venkatachalam VP. Oral submucous fibrosis--treatment with hyalase. J Laryngol Otol 1985 Jan;99(1):57-9.
[47] Anil S, Beena VT. Oral submucous fibrosis in a 12-year-old girl: case report. Pediatr Dent 1993 Mar-Apr;15(2):120-2.
[48] Haque MF, Meghji S, Nazir R, Harris M. Interferon gamma (IFN-gamma) may reverse oral submucous fibrosis. J Oral Pathol Med 2001 Jan;30(1):12-21.
[49] Fedorowicz Z, Chan Shih-Yen E, Dorri M, Nasser M, Newton T, Shi L. Interventions for the management of oral submucous fibrosis. Cochrane Database Syst Rev 2008(4):CD007156.
[50] Murlidhar V, Upmanyu G. Tobacco chewing, oral submucous fibrosis, and anaesthetic risk. Lancet 1996 Jun 29;347(9018):1840.
[51] OCIU. Profile of Head and Neck Cancers in England: Incidence, Mortality and Survival. : Oxford Cancer Intelligence Unit; 2010.
[52] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2011 Dec 15;127(12):2893-917.
[53] Moles DR, Fedele S, Speight PM, Porter SR, dos Santos Silva I. Oral and pharyngeal cancer in South Asians and non-South Asians in relation to socioeconomic deprivation in South East England. Br J Cancer 2008 Feb 12;98(3):633-5.
[54] ONS. Census: Neighbourhood Ethnic Minority Statistics. 2001 [cited; Available from:
[55] IARC. A digital manual for the early diagnosis of oral neoplasia: International Agency for Research on Cancer; 2012.
[56] Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, et al. SEER Cancer Statistics Review, 1975-2007. . 2009 [cited; Available from: http://www.seer.cancer.gov/csr/1975_2007/index.html
[57] Bessell A, Glenny AM, Furness S, Clarkson JE, Oliver R, Conway DI, et al. Interventions for the treatment of oral and oropharyngeal cancers: surgical treatment. Cochrane Database Syst Rev 2011(9):CD006205.
[58] Glenny AM, Furness S, Worthington HV, Conway DI, Oliver R, Clarkson JE, et al. Interventions for the treatment of oral cavity and oropharyngeal cancer: radiotherapy. Cochrane Database Syst Rev 2010(12):CD006387.

[59] UKNSC. Criteria for appraising the effectiveness and appropriateness of a screening programme. . 2009 [cited; Available from: http://www.screening.nhs.uk/criteria
[60] Speight P, Warnakulasuriya KA. The UK NSC Policy on Oral Cancer Screening in Adults: UK National Screening Committee; 2010.
[61] Brocklehurst PR, Baker SR, Speight PM. Factors affecting the referral of potentially malignant lesions from primary dental care: a pilot study in South Yorkshire. Prim Dent Care 2009 Jan;16(1):13-8.
[62] Downer MC, Moles DR, Palmer S, Speight PM. A systematic review of measures of effectiveness in screening for oral cancer and precancer. Oral Oncol 2006 Jul;42(6):551-60.
[63] Whited JD, Grichnik JM. The rational clinical examination. Does this patient have a mole or a melanoma? JAMA 1998 Mar 4;279(9):696-701.
[64] Speight PM, Palmer S, Moles DR, Downer MC, Smith DH, Henriksson M, et al. The cost-effectiveness of screening for oral cancer in primary care. Health Technol Assess 2006 Apr;10(14):1-144, iii-iv.
[65] Speight PM, Elliott AE, Jullien JA, Downer MC, Zakzrewska JM. The use of artificial intelligence to identify people at risk of oral cancer and precancer. Br Dent J 1995 Nov 25;179(10):382-7.
[66] Gore S. Biostatistics and the Medical Research Council. Med Res Council News 1987;36:19-20.
[67] Claxton K, Sculpher M, Drummond M. A rational framework for decision making by the National Institute For Clinical Excellence (NICE). Lancet 2002 Aug 31;360(9334):711-5.
[68] Clarkson J, Harrison JE, Ismail AI, Needleman I, Worthington HV. Evidence Based Dentistry for Effective Practice. London: Taylor & Francis Group: 2003.
[69] Johnston K, Buxton MJ, Jones DR, Fitzpatrick R. Assessing the costs of healthcare technologies in clinical trials. Health Technol Assess 1999;3(6):1-76.
[70] Brocklehurst P, Kujan O, Glenny AM, Oliver R, Sloan P, Ogden G, et al. Screening programmes for the early detection and prevention of oral cancer. Cochrane Database Syst Rev 2010(11):CD004150.
[71] Rethman MP, Carpenter W, Cohen EE, Epstein J, Evans CA, Flaitz CM, et al. Evidence-based clinical recommendations regarding screening for oral squamous cell carcinomas. Tex Dent J 2012 May;129(5):491-507.
[72] Allison P. Effectiveness of screening for oral cancer not proven. Evid Based Dent 2004;5(2):40-1.
[73] Fernandez Garrote L, Sankaranarayanan R, Lence Anta JJ, Rodriguez Salva A, Maxwell Parkin D. An evaluation of the oral cancer control program in Cuba. Epidemiology 1995 Jul;6(4):428-31.
[74] Holmstrup P, Vedtofte P, Reibel J, Stoltze K. Oral premalignant lesions: is a biopsy reliable? J Oral Pathol Med 2007 May;36(5):262-6.
[75] Moule I, Parsons PA, Irvine GH. Avoiding artefacts in oral biopsies: the punch biopsy versus the incisional biopsy. Br J Oral Maxillofac Surg 1995 Aug;33(4):244-7.
[76] Pindborg JJ, Reibel J, Holmstrup P. Subjectivity in evaluating oral epithelial dysplasia, carcinoma in situ and initial carcinoma. J Oral Pathol 1985 Oct;14(9):698-708.
[77] Scully C, Bagan JV, Hopper C, Epstein JB. Oral cancer: current and future diagnostic techniques. Am J Dent 2008 Aug;21(4):199-209.
[78] Kusukawa J, Suefuji Y, Ryu F, Noguchi R, Iwamoto O, Kameyama T. Dissemination of cancer cells into circulation occurs by incisional biopsy of oral squamous cell carcinoma. J Oral Pathol Med 2000 Aug;29(7):303-7.

[79] Holmstrup P, Vedtofte P, Reibel J, Stoltze K. Long-term treatment outcome of oral premalignant lesions. Oral Oncol 2006 May;42(5):461-74.
[80] Sciubba JJ. Improving detection of precancerous and cancerous oral lesions. Computer-assisted analysis of the oral brush biopsy. U.S. Collaborative OralCDx Study Group. J Am Dent Assoc 1999 Oct;130(10):1445-57.
[81] Lingen MW. Cause-related marketing: the uneasy alliance between altruism and corporate greed. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008 Apr;105(4):407-9.
[82] Sciubba J. Comment on "critical evaluation of diagnostic aids for the detection of oral cancer". Oral Oncol 2008 May;44(5):518-9; author reply 20-2.
[83] Eisen D. Comment on "Critical evaluation of diagnostic aids for the detection of oral cancer". Oral Oncol 2008 Oct;44(10):994-6; author reply 7-8.
[84] Bradley G, Odell EW, Raphael S, Ho J, Le LW, Benchimol S, et al. Abnormal DNA content in oral epithelial dysplasia is associated with increased risk of progression to carcinoma. Br J Cancer 2010 Oct 26;103(9):1432-42.
[85] Teh MT, Gemenetzidis E, Patel D, Tariq R, Nadir A, Bahta AW, et al. FOXM1 induces a global methylation signature that mimics the cancer epigenome in head and neck squamous cell carcinoma. PLoS One 2012;7(3):e34329.
[86] Jerjes W, Upile T, Conn B, Hamdoon Z, Betz CS, McKenzie G, et al. In vitro examination of suspicious oral lesions using optical coherence tomography. Br J Oral Maxillofac Surg 2010 Jan;48(1):18-25.
[87] Papamarkakis K, Bird B, Schubert JM, Miljkovic M, Wein R, Bedrossian K, et al. Cytopathology by optical methods: spectral cytopathology of the oral mucosa. Lab Invest 2010 Apr;90(4):589-98.
[88] Croucher R, Islam S, Jarvis MJ, Garrett M, Rahman R, Shajahan S, et al. Oral tobacco cessation with UK resident Bangladeshi women: a community pilot investigation. Health Educ Res 2003 Apr;18(2):216-23.
[89] Bhopal R. Racism, socioeconomic deprivation, and health in New Zealand. Lancet 2006 Jun 17;367(9527):1958-9.
[90] Jain RV, Mills PK, Parikh-Patel A. Cancer incidence in the south Asian population of California, 1988-2000. J Carcinog 2005 Nov 10;4:21.
[91] Grulich AE, McCredie M, Coates M. Cancer incidence in Asian migrants to New South Wales, Australia. Br J Cancer 1995 Feb;71(2):400-8.
[92] Balarajan R, Raleigh VS. Patterns of mortality among Bangladeshis in England and Wales. Ethn Health 1997 Mar-Jun;2(1-2):5-12.
[93] HSE. Health Survey for England 2004. The Health of Minority Ethnic Groups.: Department of Health; 2005.
[94] Pearson N, Croucher R, Marcenes W, O'Farrell M. Dental health and treatment needs among a sample of Bangladeshi medical users aged 40 years and over living in Tower Hamlets, UK. Int Dent J 2001 Feb;51(1):23-9.
[95] Williams SA, Gelbier S. Dentists and ethnic minority communities. Br Dent J 1989 Mar 25;166(6):194-5.
[96] Hoare T. Breast screening and ethnic minorities. Br J Cancer Suppl 1996 Sep;29:S38-41.
[97] Szczepura A. Access to health care for ethnic minority populations. Postgrad Med J 2005 Mar;81(953):141-7.
[98] McAvoy BR, Raza R. Can health education increase uptake of cervical smear testing among Asian women? BMJ 1991 Apr 6;302(6780):833-6.

[99] Sharp DJ, Peters TJ, Bartholomew J, Shaw A. Breast screening: a randomised controlled trial in UK general practice of three interventions designed to increase uptake. J Epidemiol Community Health 1996 Feb;50(1):72-6.
[100] Hoare T, Thomas C, Biggs A, Booth M, Bradley S, Friedman E. Can the uptake of breast screening by Asian women be increased? A randomized controlled trial of a linkworker intervention. J Public Health Med 1994 Jun;16(2):179-85.
[101] Kernohan EE. Evaluation of a pilot study for breast and cervical cancer screening with Bradford's minority ethnic women; a community development approach, 1991-93. Br J Cancer Suppl 1996 Sep;29:S42-6.
[102] Bell TS, Branston LK, Newcombe RG, Barton GR. Interventions to improve uptake of breast screening in inner city Cardiff general practices with ethnic minority lists. Ethn Health 1999 Nov;4(4):277-84.
[103] Ecob R, Williams R. Sampling Asian minorities to assess health and welfare. J Epidemiol Community Health 1991 Jun;45(2):93-101.
[104] Ahmed S, Rahman A, Hull S. Use of betel quid and cigarettes among Bangladeshi patients in an inner-city practice: prevalence and knowledge of health effects. Br J Gen Pract 1997 Jul;47(420):431-4.
[105] Pearson N, Croucher R, Marcenes W, O'Farrell M. Dental service use and the implications for oral cancer screening in a sample of Bangladeshi adult medical care users living in Tower Hamlets, UK. Br Dent J 1999 May 22;186(10):517-21.
[106] THPCT. Response to Scrutiny Working Group Report on Access to GP / Dentistry Services. London: Tower Hamlet Primary Care Trust; 2008.
[107] Petti S, Scully C. Oral cancer knowledge and awareness: primary and secondary effects of an information leaflet. Oral Oncol 2007 Apr;43(4):408-15.
[108] Stahl S, Meskin LH, Brown LJ. The American Dental Association's oral cancer campaign: the impact on consumers and dentists. J Am Dent Assoc 2004 Sep;135(9):1261-7.
[109] Moles DR, Downer MC, Speight PM. Meta-analysis of measures of performance reported in oral cancer and precancer screening studies. Br Dent J 2002 Mar 23;192(6):340-4; discussion 32.
[110] Onizawa K, Nishihara K, Yamagata K, Yusa H, Yanagawa T, Yoshida H. Factors associated with diagnostic delay of oral squamous cell carcinoma. Oral Oncol 2003 Dec;39(8):781-8.
[111] Elkan R, Avis M, Cox K, Wilson E, Patel S, Miller S, et al. The reported views and experiences of cancer service users from minority ethnic groups: a critical review of the literature. Eur J Cancer Care (Engl) 2007 Mar;16(2):109-21.
[112] Yellowitz J, Horowitz AM, Goodman HS, Canto MT, Farooq NS. Knowledge, opinions and practices of general dentists regarding oral cancer: a pilot survey. J Am Dent Assoc 1998 May;129(5):579-83.
[113] Kujan O, Duxbury AJ, Glenny AM, Thakker NS, Sloan P. Opinions and attitudes of the UK's GDPs and specialists in oral surgery, oral medicine and surgical dentistry on oral cancer screening. Oral Dis 2006 Mar;12(2):194-9.
[114] Decuseara G, MacCarthy D, Menezes G. Oral cancer: knowledge, practices and opinions of dentists in Ireland. J Ir Dent Assoc 2011 Aug-Sep;57(4):209-14.
[115] Seoane J, Varela-Centelles P, Tomas I, Seoane-Romero J, Diz P, Takkouche B. Continuing education in oral cancer prevention for dentists in Spain. J Dent Educ 2012 Sep;76(9):1234-40.
[116] WHO. Oral Health Surveys - Basic Methods. 4th Edition.: World Health Organisation Press: 1997.
[117] DoH. NHS Performance Indicators: February 2002: Department of Health; 2002.

[118] Kujan O, Glenny AM, Duxbury AJ, Thakker N, Sloan P. Screening programmes for the early detection and prevention of oral cancer. Cochrane Database Syst Rev 2003(4):CD004150.
[119] Petti S. Pooled estimate of world leukoplakia prevalence: a systematic review. Oral Oncol 2003 Dec;39(8):770-80.
[120] Haque MF, Hussain MA, Harris M. Prevalence of Oral Submucous Fibrosis among Bangladeshi's in Tower Hamlets. Journal of Dental Research 1997;76:1024.
[121] Mattin D. The Oral Health Status, Dental needs and Factors Affecting Utilisation of Dental Services in Asians Aged 55 Years and Over, Resident in Southampton. MSc Thesis. London: University of London; 1989.
[122] Mehrotra R, Thomas S, Nair P, Pandya S, Singh M, Nigam NS, et al. Prevalence of oral soft tissue lesions in Vidisha. BMC Res Notes 2010;3:23.
[123] Dagli RJ, Kumar S, Mathur A, Balasubrimanyam G, Duraiswamy P, Kulkarni S. Prevalence of leukoplakia, oral submucous fibrosis, papilloma and its relation with stress among green marbles mine laborers, India. Med Oral Patol Oral Cir Bucal 2008 Nov;13(11):E687-92.
[124] Gupta PC, Mehta FS, Pindborg JJ, Bhonsle RB, Murti PR, Daftary DK, et al. Primary prevention trial of oral cancer in india: a 10-year follow-up study. J Oral Pathol Med 1992 Nov;21(10):433-9.
[125] Nemes S, Jonasson JM, Genell A, Steineck G. Bias in odds ratios by logistic regression modelling and sample size. BMC Med Res Methodol 2009;9:56.
[126] Dodson A. Modern methods for diagnostic immunocytochemistry. Current Diagnostic Pathology 2002;8:113-22.
[127] Fraenkel-Conrat H, Mecham DK. The reaction of formaldehyde with proteins; demonstration of intermolecular cross-linking by means of osmotic pressure measurements. J Biol Chem 1949 Jan;177(1):477-86.
[128] Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature 2006 Jun 29;441(7097):1068-74.
[129] Pindborg JJ, Sirsat SM. Oral submucous fibrosis. Oral Surg Oral Med Oral Pathol 1966 Dec;22(6):764-79.
[130] Pindborg JJ, Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS. Oral submucous fibrosis as a precancerous condition. Scand J Dent Res 1984 Jun;92(3):224-9.
[131] Canniff JP, Harvey W, Harris M. Oral submucous fibrosis: its pathogenesis and management. Br Dent J 1986 Jun 21;160(12):429-34.
[132] Rajendran R, Sunil, Twinkle SP, Anikumar TV, Annie J. Cell death does not herald epithelial involution ("atrophy") in oral sub mucous fibrosis: a TEM study. Indian J Dent Res 2004 Jan-Mar;15(1):13-9.
[133] Bloor BK, Tidman N, Leigh IM, Odell E, Dogan B, Wollina U, et al. Expression of keratin K2e in cutaneous and oral lesions: association with keratinocyte activation, proliferation, and keratinization. Am J Pathol 2003 Mar;162(3):963-75.
[134] Moll R, Franke WW, Volc-Platzer B, Krepler R. Different keratin polypeptides in epidermis and other epithelia of human skin: a specific cytokeratin of molecular weight 46,000 in epithelia of the pilosebaceous tract and basal cell epitheliomas. J Cell Biol 1982 Oct;95(1):285-95.
[135] Waseem A, Gough AC, Spurr NK, Lane EB. Localization of the gene for human simple epithelial keratin 18 to chromosome 12 using polymerase chain reaction. Genomics 1990 Jun;7(2):188-94.
[136] Machesney M, Tidman N, Waseem A, Kirby L, Leigh I. Activated keratinocytes in the epidermis of hypertrophic scars. Am J Pathol 1998 May;152(5):1133-41.

[137] Wong P, Coulombe PA. Loss of keratin 6 (K6) proteins reveals a function for intermediate filaments during wound repair. J Cell Biol 2003 Oct 27;163(2):327-37.
[138] Kim S, Wong P, Coulombe PA. A keratin cytoskeletal protein regulates protein synthesis and epithelial cell growth. Nature 2006 May 18;441(7091):362-5.
[139] Bloor BK, Seddon SV, Morgan PR. Gene expression of differentiation-specific keratins (K4, K13, K1 and K10) in oral non-dysplastic keratoses and lichen planus. J Oral Pathol Med 2000 Sep;29(8):376-84.
[140] Bloor BK, Seddon SV, Morgan PR. Gene expression of differentiation-specific keratins in oral epithelial dysplasia and squamous cell carcinoma. Oral Oncol 2001 Apr;37(3):251-61.
[141] Wu C. Heat shock transcription factors: structure and regulation. Annu Rev Cell Dev Biol 1995;11:441-69.
[142] Vaidya MM, Sawant SS, Borges AM, Ogale SB, Bhisey AN. Cytokeratin expression in precancerous lesions of the human oral cavity. Oral Oncol 1998 Jul;34(4):261-4.
[143] Ranganathan K, Kavitha R, Sawant SS, Vaidya MM. Cytokeratin expression in oral submucous fibrosis--an immunohistochemical study. J Oral Pathol Med 2006 Jan;35(1):25-32.
[144] Waseem A, Dogan B, Tidman N, Alam Y, Purkis P, Jackson S, et al. Keratin 15 expression in stratified epithelia: downregulation in activated keratinocytes. J Invest Dermatol 1999 Mar;112(3):362-9.
[145] Ku NO, Zhou X, Toivola DM, Omary MB. The cytoskeleton of digestive epithelia in health and disease. Am J Physiol 1999 Dec;277(6 Pt 1):G1108-37.
[146] Bloor BK, Su L, Shirlaw PJ, Morgan PR. Gene expression of differentiation-specific keratins (4/13 and 1/10) in normal human buccal mucosa. Lab Invest 1998 Jul;78(7):787-95.
[147] Hansson A, Bloor BK, Haig Y, Morgan PR, Ekstrand J, Grafstrom RC. Expression of keratins in normal, immortalized and malignant oral epithelia in organotypic culture. Oral Oncol 2001 Jul;37(5):419-30.
[148] Waseem A, Lane EB, Harrison D, Waseem N. A keratin antibody recognizing a heterotypic complex: epitope mapping to complementary locations on both components of the complex. Exp Cell Res 1996 Mar 15;223(2):203-14.
[149] Waseem A, White K, Waseem NH. Identification of a novel keratin epitope: evidence for association between non-helical sub-domains L12 during filament assembly. Int J Biochem Cell Biol 1997 Jul;29(7):971-83.
[150] Su L, Morgan PR, Lane EB. Keratin 14 and 19 expression in normal, dysplastic and malignant oral epithelia. A study using in situ hybridization and immunohistochemistry. J Oral Pathol Med 1996 Jul;25(6):293-301.
[151] van der Velden LA, Manni JJ, Ramaekers FC, Kuijpers W. Expression of intermediate filament proteins in benign lesions of the oral mucosa. Eur Arch Otorhinolaryngol 1999;256(10):514-9.
[152] Elie N, Plancoulaine B, Signolle JP, Herlin P. A simple way of quantifying immunostained cell nuclei on the whole histologic section. Cytometry A 2003 Nov;56(1):37-45.
[153] Mackenzie IC. The role of epithelial-mesenchymal interactions in epithelial migration and differentiation. J Periodontal Res 1984 Nov;19(6):656-60.
[154] Mueller MM, Fusenig NE. Tumor-stroma interactions directing phenotype and progression of epithelial skin tumor cells. Differentiation 2002 Dec;70(9-10):486-97.

[155] Chinnathambi S, Tomanek-Chalkley A, Ludwig N, King E, DeWaard R, Johnson G, et al. Recapitulation of oral mucosal tissues in long-term organotypic culture. Anat Rec A Discov Mol Cell Evol Biol 2003 Feb;270(2):162-74.
[156] Mackenzie IC, Hill MW. Maintenance of regionally specific patterns of cell proliferation and differentiation in transplanted skin and oral mucosa. Cell Tissue Res 1981;219(3):597-607.
[157] Andriessen MP, Niessen FB, Van de Kerkhof PC, Schalkwijk J. Hypertrophic scarring is associated with epidermal abnormalities: an immunohistochemical study. J Pathol 1998 Oct;186(2):192-200.
[158] Moore RJ, Owens DM, Stamp G, Arnott C, Burke F, East N, et al. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. Nat Med 1999 Jul;5(7):828-31.
[159] Lane EB, Alexander CM. Use of keratin antibodies in tumor diagnosis. Semin Cancer Biol 1990 Jun;1(3):165-79.
[160] Ogden GR, Lunny D, Lane EB. Keratin antigen retrieval in oral mucosal biopsies using microwave processing. Histochem J 1996 Dec;28(12):875-82.
[161] Waseem A, Karsten U, Leigh IM, Purkis P, Waseem NH, Lane EB. Conformational changes in the rod domain of human keratin 8 following heterotypic association with keratin 18 and its implication for filament stability. Biochemistry 2004 Feb 10;43(5):1283-95.
[162] Leers MP, Kolgen W, Bjorklund V, Bergman T, Tribbick G, Persson B, et al. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. J Pathol 1999 Apr;187(5):567-72.
[163] Ditzel HJ, Strik MC, Larsen MK, Willis AC, Waseem A, Kejling K, et al. Cancer-associated cleavage of cytokeratin 8/18 heterotypic complexes exposes a neoepitope in human adenocarcinomas. J Biol Chem 2002 Jun 14;277(24):21712-22.
[164] Lindberg K, Rheinwald JG. Suprabasal 40 kd keratin (K19) expression as an immunohistologic marker of premalignancy in oral epithelium. Am J Pathol 1989 Jan;134(1):89-98.
[165] Su L, Morgan PR, Lane EB. Protein and mRNA expression of simple epithelial keratins in normal, dysplastic, and malignant oral epithelia. Am J Pathol 1994 Dec;145(6):1349-57.
[166] Tomic-Canic M, Komine M, Freedberg IM, Blumenberg M. Epidermal signal transduction and transcription factor activation in activated keratinocytes. J Dermatol Sci 1998 Jul;17(3):167-81.
[167] Jiang CK, Flanagan S, Ohtsuki M, Shuai K, Freedberg IM, Blumenberg M. Disease-activated transcription factor: allergic reactions in human skin cause nuclear translocation of STAT-91 and induce synthesis of keratin K17. Mol Cell Biol 1994 Jul;14(7):4759-69.
[168] Bonnekoh B, Huerkamp C, Wevers A, Geisel J, Sebok B, Bange FC, et al. Up-regulation of keratin 17 expression in human HaCaT keratinocytes by interferon-gamma. J Invest Dermatol 1995 Jan;104(1):58-61.
[169] Radoja N, Stojadinovic O, Waseem A, Tomic-Canic M, Milisavljevic V, Teebor S, et al. Thyroid hormones and gamma interferon specifically increase K15 keratin gene transcription. Mol Cell Biol 2004 Apr;24(8):3168-79.
[170] Mikami T, Cheng J, Maruyama S, Kobayashi T, Funayama A, Yamazaki M, et al. Emergence of keratin 17 vs. loss of keratin 13: their reciprocal immunohistochemical profiles in oral carcinoma in situ. Oral Oncol 2011 Jun;47(6):497-503.

[171] Wei KJ, Zhang L, Yang X, Zhong LP, Zhou XJ, Pan HY, et al. Overexpression of cytokeratin 17 protein in oral squamous cell carcinoma in vitro and in vivo. Oral Dis 2009 Jan;15(1):111-7.
[172] Ogden GR, Chisholm DM, Leigh IM, Lane EB. Cytokeratin profiles in dyskeratosis congenita: an immunocytochemical investigation of lingual hyperkeratosis. J Oral Pathol Med 1992 Sep;21(8):353-7.
[173] Field EA, Morrison T, Darling AE, Parr TA, Zakrzewska JM. Oral mucosal screening as an integral part of routine dental care. Br Dent J 1995 Oct 7;179(7):262-6.
[174] Jullien JA, Zakrzewska JM, Downer MC, Speight PM. Attendance and compliance at an oral cancer screening programme in a general medical practice. Eur J Cancer B Oral Oncol 1995 May;31B(3):202-6.
[175] Jullien JA, Downer MC, Zakrzewska JM, Speight PM. Evaluation of a screening test for the early detection of oral cancer and precancer. Community Dent Health 1995 Mar;12(1):3-7.
[176] Downer MC, Evans AW, Hughes Hallet CM, Jullien JA, Speight PM, Zakrzewska JM. Evaluation of screening for oral cancer and precancer in a company headquarters. Community Dent Oral Epidemiol 1995 Apr;23(2):84-8.

APPENDIX

doi: 10.1111/j.1600-0714.2007.00609.x

J Oral Pathol Med (2008) 37: 211–220

www.blackwellmunksgaard.com/jopm

An altered keratinocyte phenotype in oral submucous fibrosis: correlation of keratin K17 expression with disease severity

Anand Lalli[1], Wanninayake M. Tilakaratne[1,3], Anura Ariyawardana[4], Caroline Fitchett[1], Irene M. Leigh[2,*], Eleni Hagi-Pavli[1], Alan T. Cruchley[1], E. Kenneth Parkinson[1], Muy-Teck Teh[1], Farida Fortune[1], Ahmad Waseem[1]

[1]*Programme in Muco-Cutaneous Oncology, Centre for Clinical and Diagnostic Oral Sciences, Institute of Cell and Molecular Sciences, London, UK;* [2]*Centre for Cutaneous Research; Barts and The London, Queen Mary's School of Medicine and Dentistry, Institute of Cell and Molecular Sciences, London, UK;* [3]*Department of Oral Pathology, University of Peradeniya, Peradeniya, Sri Lanka;* [4]*Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya, Peradeniya, Sri Lanka*

Oral submucous fibrosis (OSF) is characterized by abnormal collagen metabolism in the submucosal connective tissue. Its influence on the overlying epithelium is not known but about 14% of OSF cases undergo malignant transformation to squamous cell carcinoma indicating association with abnormality of the epithelium. Here, we have defined the keratin expression profile, by immunohistochemistry and quantitative image analysis, using a panel of 22 anti-keratin monoclonal antibodies on 28 OSF samples. We observed an increase of K1 and K10 in the suprabasal layers, induction of K6 in the basal layer and complete loss of K19 in the epithelium. Furthermore, there was increased K17 expression in the suprabasal layers, which correlated with disease severity. In a subset of the most severe OSF cases (14%), K17 expression was completely lost in the basal layer which might define them to be at most risk to undergo malignant transformation. There was no detectable expression of K8, K18, K7 and K9 and the expression of K4, K13, K14, K15 and K16 did not change in OSF. We propose that the altered keratin profiles could be useful as histological diagnostic markers and provide important insights into the pathogenesis of the disease and its predisposition to malignancy.

J Oral Pathol Med (2008) **37:** 211–220

Correspondence: Ahmad Waseem, Centre for Clinical and Diagnostic Oral Sciences, Institute of Cell and Molecular Sciences, 4 Newark Street, London E1 2AT, UK. Tel: 0044-207-8822387, Fax: 0044-207-8827153, E-mail: a.waseem@qmul.ac.uk
*Present address: College of Medicine, Dentistry and Nursing, University of Dundee, Level 10, Ninewells Hospital & Medical School, Dundee DD1 9SY, UK.
Accepted for publication July 4, 2007

Keywords: epithelium; immunohistochemistry; keratin; pre-malignancy

Introduction

Oral submucous fibrosis (OSF) is a chronic debilitating pre-malignant condition affecting millions of individuals worldwide. Incidence varies between countries but OSF is most commonly seen in the Asian subcontinent. Malignancy develops in about 14% of OSF lesions (1) and contributes to oral cancer being the eighth most common cancer worldwide and one of the three commonest cancers in South-Central Asia (2). Although the aetiology of OSF is largely unknown there is a relationship between the use of areca nut extract and development of OSF prompting the World Health Organization to classify areca nut as a group I carcinogen (3). However, OSF pathogenesis is likely to be multifactorial as only a small proportion of the nut users actually suffer from this condition.

Clinically, OSF is characterized by progressive loss of elasticity of the oropharyngeal mucosa and atrophy of the oral epithelium with resultant restricted mouth opening, reduced tongue mobility and sensitivity to spicy food. The characteristic histological change in OSF is progressive collagenous fibrosis and hyalinization of the subepithelial connective tissue. As the disease is considered to be mesenchymal in origin, research in the literature has focussed on the cellular and biochemical events that take place in the connective tissue (4, 5). Despite these advances the pathogenesis remains to be elucidated. At present nothing is known about the effects of fibrosis on the overlying epithelium at the molecular level but histology shows

the presence of epithelial abnormalities. Dysplasia is a feature in 26% of OSF lesions (6), which is consistent with the high rate of malignant transformation. Additionally, epithelial atrophy occurs in 87% and keratinizing metaplasia in 67% of cases (7). Given these morphological changes it is plausible that the epithelium may play a role in inducing the connective tissue changes seen in OSF and is certainly involved in the malignant changes that occur in a significant number of cases.

Oral epithelium is formed of stratified layers of keratinocytes which express matched pairs of type I and type II keratin polypeptides in a tissue- and differentiation-specific manner (8, 9). For example, all stratified epithelia express keratins K5 and K14/K15 in their basal layer but the suprabasal layers express different pairs. In non-keratinizing epithelia such as buccal mucosa the suprabasal keratinocytes express K4 and K13, whereas the keratinizing epithelia such as gingivae and hard palate express K1 and K10 as the predominant keratins. Keratin gene expression is extremely sensitive to pathological signalling and altered keratinocyte phenotypes have been described using keratin gene expression both in cutaneous and in oral diseases (10, 11).

In this study, we describe an OSF-specific keratinocyte phenotype with a different keratin expression profile from other cutaneous and oral diseases. The extensive changes in expression were present in the basal layer and also affected the suprabasal keratinocytes. These changes in keratin gene expression provide an insight into the mechanisms of OSF development and its conversion to oral squamous cell carcinoma.

Materials and methods

Tissue samples

Samples of site-matched normal (six patients) and OSF tissue (28 patients) were collected with ethical committee approval for use in this study. Four normal samples and 22 OSF biopsies were obtained from normally non-keratinizing buccal mucosa and the remainder from keratinized sites such as the tongue and gingivae (Supplementary Table S1 shows the biopsy site of each sample and is submitted for the online version of this journal at http://www.blackwell-synergy.com/). OSF was diagnosed by clinical presentation and confirmed by histological evaluation. Biopsy specimens were snap frozen and serial 6 μm sections cut in a cryostat (Bright Instruments, Cambridge, UK).

Antibodies and immunohistochemistry

Twenty-two different monoclonal mouse antibodies (mAbs) were obtained, either from commercial sources or raised in house by culturing the respective hybridomas (Table 1). Three layer indirect immunohistochemical staining was performed on frozen tissue sections utilizing the DAKO LSAB+ kit (Dako, Cambridgeshire, UK). Colour was developed using 3,3′-diaminobenzidine (DAB; Vector Labs, Peterborough, UK) with a haematoxylin counterstain.

Table 1 Mouse monoclonal antibodies used for immunohistochemical characterization of keratin expression in oral submucous fibrosis

Clone	*Specificity*	*Working Dilution*	*Supplier*
LHK1	K1	1:500	In house
LHK2e	K2	–	In house
6B10	K4	1:10	Sigma-Aldrich, St Louis, MO, USA
LHK6B	K6	1:100	In house
LP5 K	K7	–	In house
LE41	K8	–	In house
Ks9.20 + Ks9.28	K9	1:10	Progen, Heidelberg, Germany
DE-K10	K10	1:50	Neomarkers, Fremont, CA, USA
RKSE60	K10	1:10	Biogenesis, Poole, UK
LHP2	K10	–	In house
1C7	K13	–	In house
1C7 + 2D7	K13	1:10	Neomarkers
LL001	K14	1:500	in house
LHK15	K15	1:500	in house
LL025	K16	1:500	in house
E3	K17	1:10	DAKO, Glostrup, Denmark
LP34	K6 + K18	1:500	In house
LE61	K18	1:100	In house
LE65	K18	1:10	In house
CY-90	K18	1:10	Sigma-Aldrich
LP2 K	K19	1:100	In house
RCK108	K19	1:100	DakoCytomation

Data analysis

The staining data were analysed by visual assessment and quantitated by image analysis. Each slide was visually assessed, independently by two observers, and those showing positive staining were graded on a three-point equally weighted scale (+, ++ or +++) for staining in the basal layer and the suprabasal layers. A cumulative staining quotient (CSQ) was then calculated for each antibody, in normal and OSF samples (Table 2):

$$\textit{Cumulative staining quotient (CSQ)} = \frac{\textit{Number of (+) in all samples}}{\textit{Number of samples}} \times 100$$

Haematoxylin and eosin-stained OSF lesions were graded as early, intermediate or advanced based on the extent of fibrosis and degree of inflammatory infiltrate (12) and the histopathological findings correlated with keratin staining.

Slides were photographed on a Microphot-FXA microscope with Coolpix 990 camera (Nikon, Tokyo, Japan) and the images assembled using ADOBE PHOTOSHOP CS2 v9.0.2 (Adobe Systems Inc., San Jose, CA, USA).

For quantification of staining showing a different pattern in normal and OSF samples, pixel analysis was performed on the digital images, using a modification of the approach described earlier (13). Photographs were taken at 100× magnification with identical optical and digital zoom camera settings. In PHOTOSHOP, the epithelium was carefully selected from the tissue section using the Lasso tool and the total pixel count in the selected

Table 2 CSQ of immunostaining in normal and OSF

	Cumulative staining quotient (%)			
	Basal		*Suprabasal*	
Keratin	*Normal*	*OSF*	*Normal*	*OSF*
K1 (all biopsy sites)	0	0	33	65
K1 (non-keratinized sites only)	0	0	17	65
K1 (keratinized sites only)	0	0	67	67
K10[a] (all biopsy sites)	0	0	33	69
K10[a] (non-keratinized sites only)	0	0	17	62
K10[a] (keratinized sites only)	0	0	67	67
K6	0	70	94	87
K16	0	0	94	92
K4	0	0	94	95
K13[a]	0	0	94	96
K2	0	0	11	1
K14	100	100	100	96
K15	100	99	0	0
K17	61	58	17	44
K19[a] (all samples)	22	0	0	0
K19[a] (non-keratinized sites only)	33	0	0	0
K19[a] (keratinized sites only)	0	0	0	0

[a]Results for multiple monoclonal antibodies were identical and have been combined for clarity.
CSQ, cumulative staining quotient; OSF, oral submucous fibrosis

area recorded from the Histogram palette (Fig. 3). For keratinized samples the superficial keratin layer was not selected as part of the epithelium as it is often poorly retained in frozen sections and the dense keratinization in these squames may obscure antibody-binding sites (11). A new image was created by cutting and pasting the selected epithelium and from this the basal and suprabasal compartments carefully delineated by the Lasso tool to give the pixel count for each segment of the epithelium. To separate brown DAB staining from the blue haematoxylin counterstain a colour range selection was performed using the same sampled colour with RGB values of 160, 100 and 100, respectively, as confirmed in the Colour palette of the software. The range of colours selected was set to 100 on the Fuzziness slider which selects other parts of the image by the degree their colour is related to the sample colour. To confirm that all DAB-stained sections of the image were selected, these pixels were cut from the image to leave just the counterstained areas. Any DAB-stained areas remaining when the selected pixels were removed, for example, with very dark brown staining, were manually selected and employed for the evaluation using the Plus Eyedropper tool of the software. The DAB pixel count in the basal and the suprabasal compartments of the epithelium was recorded and calculated as a percentage of the total basal or suprabasal pixel count (that will include DAB and haematoxylin). Quantification in this manner permitted use of the Mann–Whitney *U*-test to determine statistically significant differences in the DAB staining between normal and diseased samples. The validity of the pixel analysis was confirmed by correlation coefficient $r = 0.9$ ($P < 0.001$; Fig. 4) with our visual assessment grading, even with all non-stained basal or suprabasal compartments of the epithelium disregarded.

Results

Where available immunohistochemical staining was performed with several different mAbs raised against the same keratin, to ensure reproducibility of the staining pattern, and in each case comparable staining was observed (Table S1).

Suprabasal keratins (K1/K10, K2, K4/K13, K6/K16 and K9)

K16 staining was entirely suprabasal with similar expression in all OSF and normal samples confirmed by a CSQ of 92% and 94%, respectively (Fig. 1a,b and Table 2). K6 was also strongly suprabasal in normal epithelia with no expression in the basal layer (Fig. 1c) whilst in OSF K6 was detectable in both the basal and the suprabasal layers (Fig. 1d). This *increase in expression* in the basal layer of OSF epithelium was highly significant ($P < 0.001$) whilst the suprabasal K6 expression was unaffected (Table S1).

K2 was detected in a few suprabasal keratinocytes in two of six normal tissues, which originated from the tongue and gingivae with no normal buccal mucosa samples positively stained. One of 28 OSF samples was weakly positive with a suprabasal expression pattern similar to that seen in normal oral keratinized samples. This originated from the tongue and none of the other OSF samples from keratinized biopsy sites was positive indicating a potential reduction in K2 expression although the number of relevant samples was limited (Table S1).

K1 was expressed in the suprabasal layers of two of four normal buccal epithelia as well as the two biopsies from keratinized sites in the oral cavity (Table S1 and Fig. 1e). K10 staining mirrored K1 in all samples. All OSF tissues from both keratinized and non-keratinized biopsy sites were strongly K1/K10-positive (Fig. 1f). This increase was highly significant ($P < 0.001$) for non-keratinized site-matched (buccal) samples whilst normally keratinized sites appear qualitatively unaffected with a CSQ of 67% for both K1 and K10 in the suprabasal layers although the number of samples was limited (Table 2).

All OSF and normal tissue samples stained comparably for K4/K13 in the suprabasal layers (Fig. 1g,h) and none expressed the palmo-plantar epidermis-specific K9.

Basal keratins (K14, K15, K17 and K19)

K17 was detected in the basal layer of all normal samples but additional suprabasal staining was only present in 50% of cases (Figs 2a,c and 5a). All OSF samples expressed K17 and although the staining varied considerably in intensity and distribution (Figs 2b,d and 5a) there was increased K17 in the suprabasal layers ($P < 0.01$) and no change in basal expression. When correlated with histological grading the suprabasal staining was most significantly upregulated in the most severe samples ($P < 0.01$; Fig. 6). The intermediate classified samples returned weaker evidence of a difference between normal and OSF ($P < 0.05$) and for the

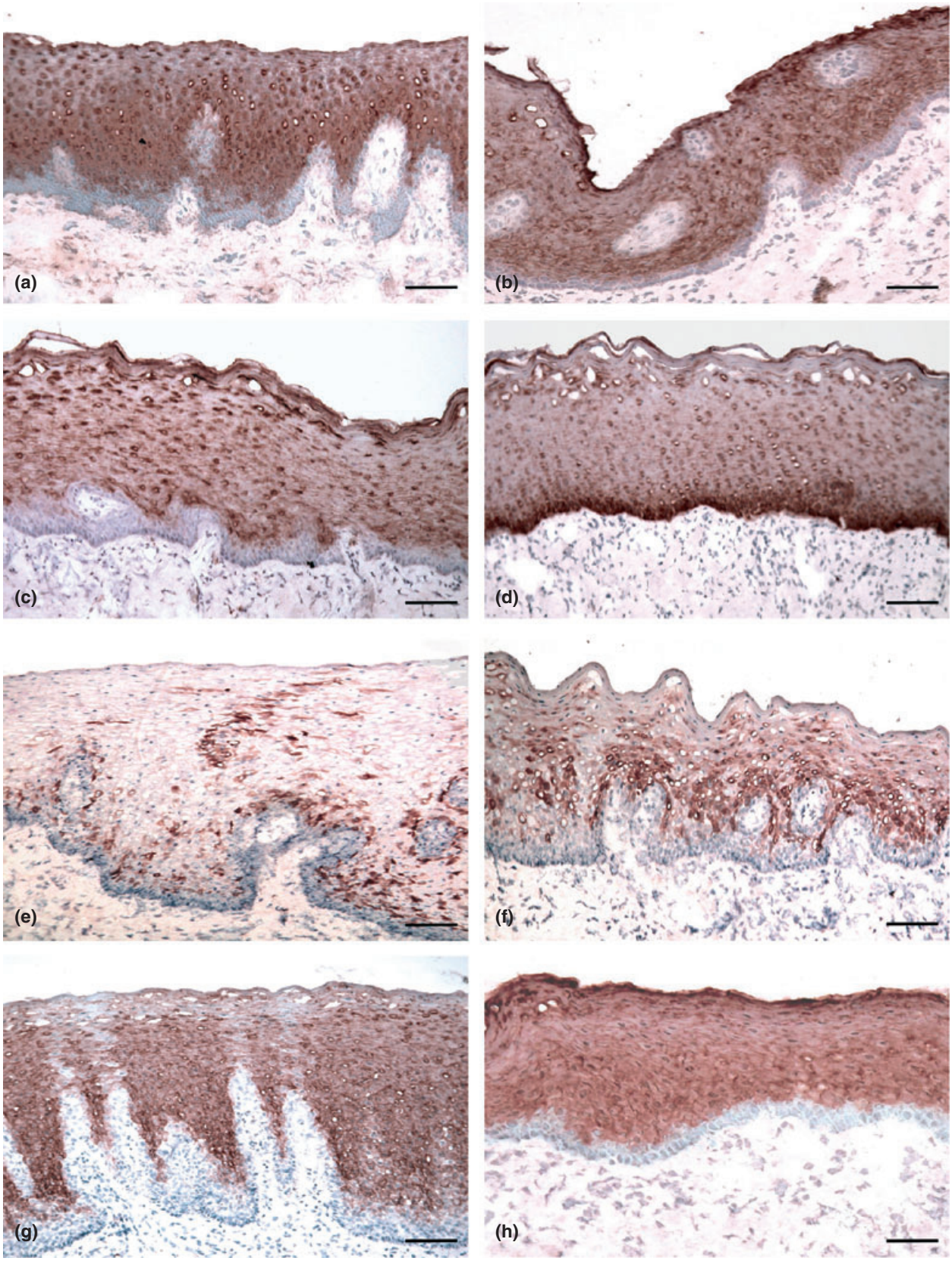


Figure 1 Immunohistochemical staining of normal oral (a, c, e and g) and oral submucous fibrosis lesional (b, d, f and h) epithelium for the suprabasal keratins. K16 (a and b), K6 (c and d), K1 (e and f) and K13 (g and h). Bar = 50 mm.

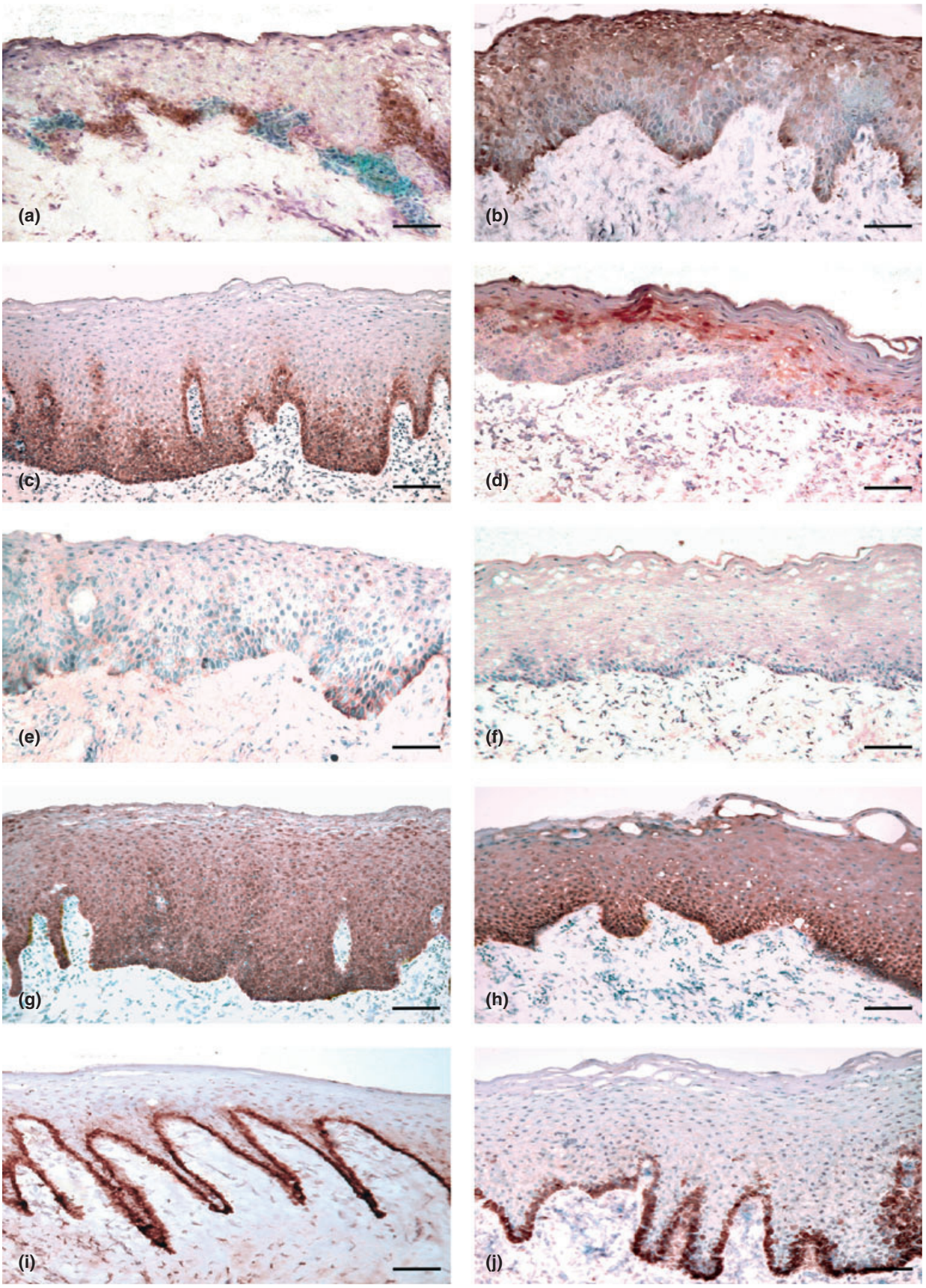


Figure 2 Immunohistochemical staining of normal oral (a, c, e, g and i) and oral submucous fibrosis lesional (b, d, f, h and j) epithelium for the basal keratins. K17 [(a) and (b) showing isolated basal and suprabasal staining whilst (c) and (d) show both basal and suprabasal staining patterns], K19 (e and f), K14 (g and h) and K15 (i and j). Bar = 50mm.

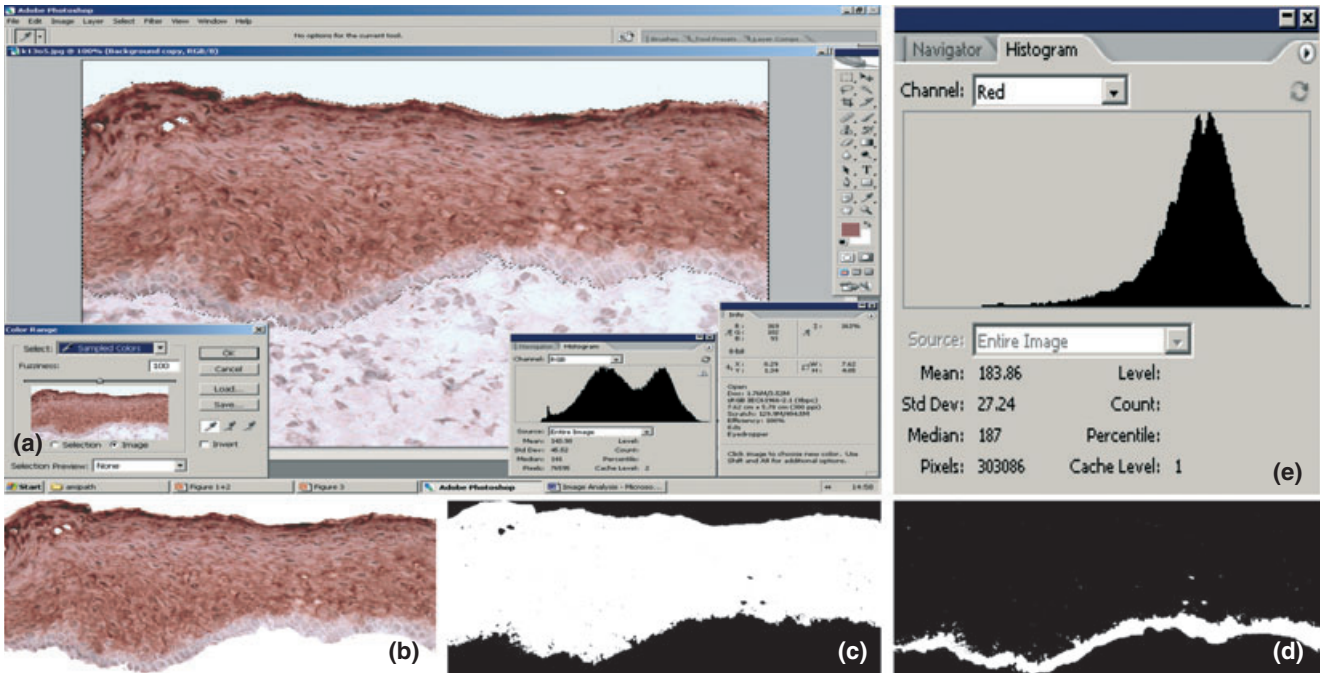


Figure 3 Quantitative analysis of 3,3′-diaminobenzidine (DAB)-immunostained and haematoxylin-counterstained tissue images. (a) Original images imported into ADOBE PHOTOSHOP CS2 v9.0.2 (Adobe Systems Inc.), (b) with epithelium delineated and (c) DAB staining specifically selected or (d) haematoxylin counterstain selected by adjustment of 'colour selection' properties. (e) The pixel count of the selected area is given in the Histogram palette.

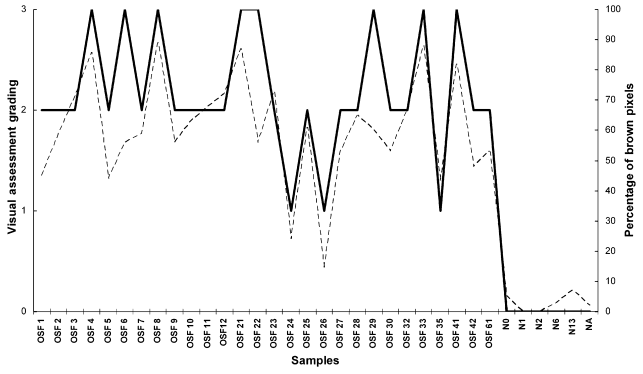


Figure 4 Correlation between visual assessment grading and image analysis of 3,3′-diaminobenzidine immunostaining for K6 in the basal layer of oral submucous fibrosis and normal oral epithelium ($r = 0.9$). Dotted line represents image analysis and the solid line represents visual assessment.

early OSF there was no significant difference to suprabasal K17 expression in normal epithelium ($P > 0.05$). Basal K17 expression did not appear to correlate with histological OSF grading.

K19 staining was discontinuous and limited to the basal layer of non-keratinized normal epithelia (Fig. 2e) with no expression in normal keratinized epithelium. There was also no expression of K19 in any OSF samples whether from keratinized or non-keratinized sites (Fig. 2f) suggesting a highly significant reduction in site-matched non-keratinized epithelia ($P < 0.001$; Fig. 5d) compared with the normal controls.

K14 staining was present homogenously throughout the basal and suprabasal layers (Fig. 2g,h) whilst K15 was detected in the entire basal layer (Fig. 2i,j) of both normal and OSF samples implying the disease did not influence these two keratins.

Simple epithelial keratins (K7, K8 and K18)

No staining for K7, K8, K18 or the K8/K18 complex was observed in normal or OSF samples.

Discussion

Epithelial–mesenchymal interactions play vital roles in keratinocyte differentiation, proliferation, migration and invasion (14, 15). Alterations in keratin expression as a result of changes in the mesenchyme have also been reported using both *in vitro* (16) and *in vivo* models (17). In OSF an imbalance of collagen metabolism causes excessive deposition of collagen leading to abnormal mesenchyme, which could affect the overlying epithelium. Two known benign connective tissue disorders of the epidermis, hypertrophic (HTS) and keloid scars, are also characterized by abnormal collagen metabolism with considerable similarities to OSF (18, 19). We and others have shown that the pathogenesis of HTS and keloids involves perturbed epithelial–mesenchymal interactions with abnormality of keratinocyte phenotype in the epidermis (11, 18, 19). Therefore, we hypothesize that keratinocytes in OSF epithelium could be altered, which would implicate them in the disease pathogenesis, as well as their predisposition to oral cancer.

Hyperkeratinization of OSF epithelium

In OSF, compared to non-keratinized normal epithelium, there is an increased suprabasal expression of

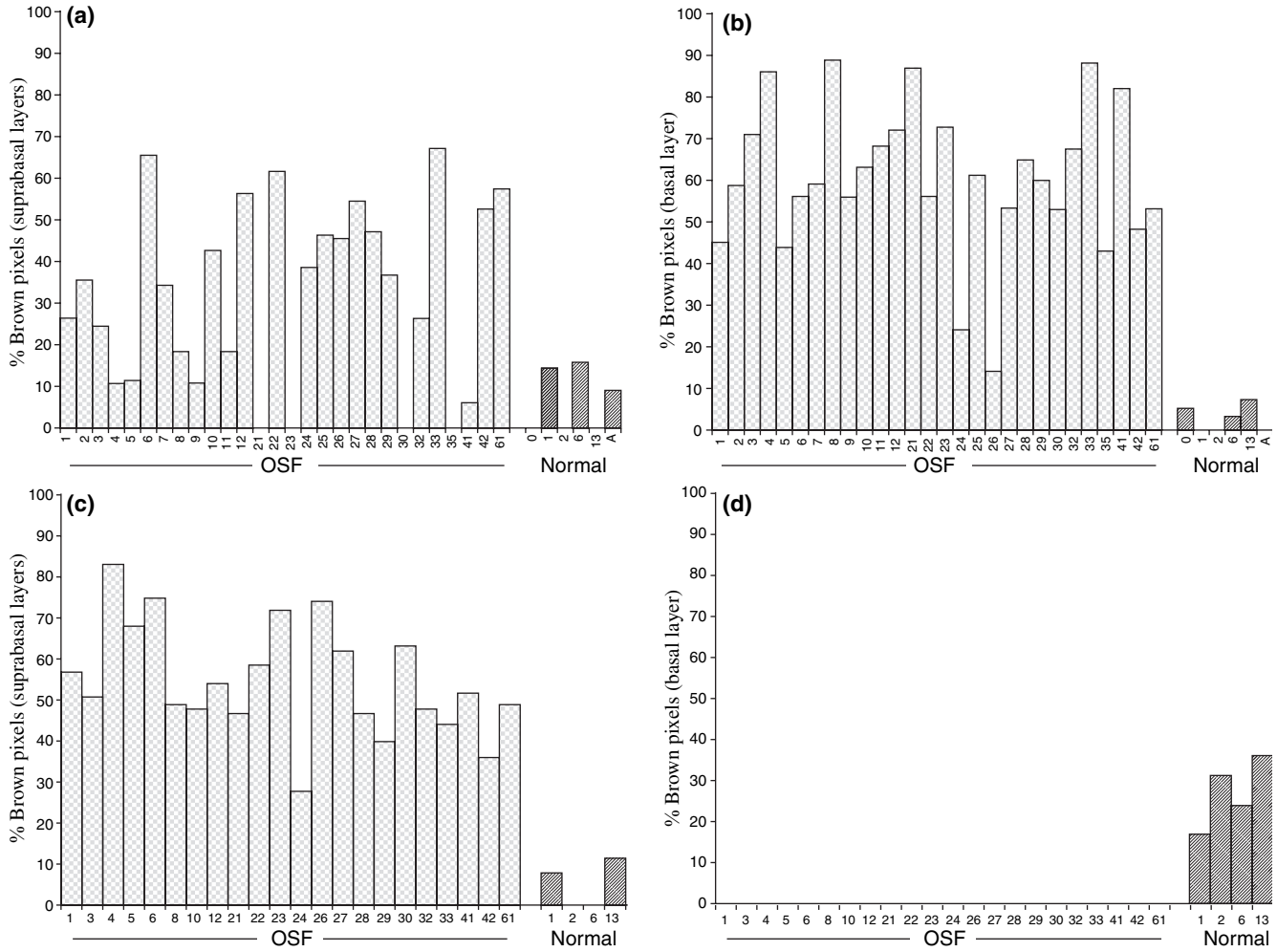


Figure 5 Quantification of keratin expression by pixel analysis of 3,3′-diaminobenzidine staining in oral submucous fibrosis (OSF) and normal epithelium. (a) Increased K17 expression in the suprabasal layers of all 28 OSF samples (keratinized and non-keratinized) compared to six normal and (b) increased K6 in the basal layer in the same samples, (c) increased suprabasal K1 and (d) basal K19 reduced expression in 22 OSF samples from non-keratinized biopsy sites compared to four non-keratinized normal oral epithelia. OSF samples are shown by dotted bars and normal by hashed bars.

K1/K10 (Fig. 5c) which is a feature of oral hyperkeratotic lesions (11). Keratinized normal oral epithelium also shows similar expression of K1/K10 indicating this to be a specific molecular marker of keratinization (20). The histologically apparent keratosis in many OSF lesions is therefore consistent with the increase in K1/K10 expression. Increased K1/K10 expression has also been linked with K2 upregulation in oral dysplastic lesions which is distinct from the keratinization seen in oral lichen planus (OLP). In OLP, keratosis K1/K10 upregulation occurs independently of K2 induction (11). OSF mirrors OLP in that K1/K10 expression is upregulated without a concomitant increase in K2 despite the presence of dysplasia. OLP is an autoimmune disorder therefore the similarities in K1/K10 and K2 regulation seen in OSF may indicate an immunological pathogenesis although it is also believed that keratinization in OSF is due to direct mechanical trauma from the course fibres of the areca nut (21). OLP is histologically characterized by a dense subepithelial band of lymphocytes, which produces a massive inflammatory infiltrate with resultant high levels of proinflammatory cytokines that induce abnormal keratinocyte differentiation (11). Whilst OSF lesions do not show this characteristic subepithelial infiltrate the dense fibrosis and reduced vasculature of OSF subepithelial tissues may allow for long-term build-up of inflammatory cytokines which can then affect the epithelium in a similar manner to OLP. This should also provide a mechanism for malignant change as persistent tissue inflammation is implicated in carcinogenesis(22).

Specific induction of K6 in the basal keratinocytes of OSF epithelium

In OSF, we observed increased K6 expression in the basal layer of the epithelium but no change in the constitutive basal keratins K14 and K15 (Figs 2g–j and 5b). The influence of OSF pathology on K5 expression could not be determined as a well-characterized mAb, suitable for frozen sections, was not available. Commercially available anti-K5 antibody XM26 (Novocastra, Newcastle, UK) has been used on

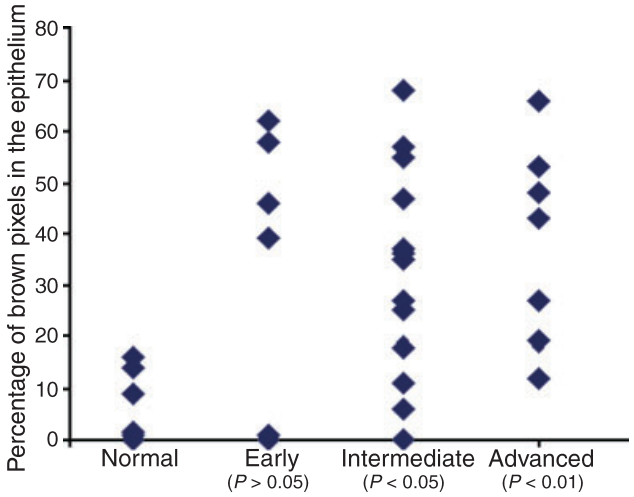


Figure 6 Suprabasal expression of K17 in oral submucous fibrosis epithelium shown by image analysis of 3,3′-diaminobenzidine immunostaining and correlated with histological grading of disease severity. No statistically significant difference is measurable between early lesions and normal epithelium but increased K17 is evident with intermediate pathology and advanced lesions display highly significant K17 suprabasal increased expression.

formalin-fixed sections but with frozen tissue this mAb did not react. This confirms that it may not be possible to compare frozen and formalin-fixed samples for keratin staining using the same mAb (23, 24). The only previous immunohistological study of keratin expression in OSF used formalin-fixed samples (25) and this may explain some of their anomalous expression patterns such as suprabasal expression of the basal-specific K5 and lack of K14 expression in 80% of samples. Alternatively, this abnormal staining could be a feature of the mAbs employed and it is unclear whether site-matched normal and OSF tissues were used in their study. Furthermore, they did not use antibodies against K6, K17 and K19, the keratins we found to be the most influenced by OSF pathology.

Induction of K6 expression in the basal layer of OSF epithelium creates a situation whereby a type II keratin is switched on without the expression of its normal type I partner K16. In non-keratinized epithelium heterogenous basal expression of K6 but not K16 has been reported previously (26) but only in the rete-ridges, using anti-K6 KA12 antibody. As no photograph of the staining was published, it was difficult to critically evaluate the reactivity. None of our normal oral tissues expressed K6 in the basal layer which is consistent with the literature using LHK6B (27) but the pattern of K6 expression in other pre-cancerous oral dysplasias is not known. Interestingly, staining observed with LP34 was entirely suprabasal in both OSF and normal tissue and this antibody is commercially marketed for the detection of K6 and K18 (Cancer Research Technology, London, UK) or K5, K6 and K18 (Acris, Hiddenhausen, Germany), indicating its precise reactivity is yet to be established. We have shown with multiple mAbs (LE61, LE65 and CY-90) that K8/K18 complex or K18 alone are not detectable in these samples, implying LP34 staining may be K6-specific. However, as K6 is present in the basal layer of OSF epithelium (Fig. 1d), the lack of LP34 reactivity suggests that this mAb may not recognize K6 either. If LP34 was reacting with K5 then staining would be observed in the basal layer, but this was absent in normal samples indicating that LP34 may not also detect K5. The LP34 staining in normal and OSF epithelium could be from cross-reactivity with another, as yet unidentified suprabasal keratin, in a manner similar to anti-K14 LL001 antibody which stains both basal and suprabasal keratinocytes in normal buccal epithelium (Fig. 2g), when the K14 mRNA expression is restricted to the basal layer (10). Alternatively, it is conceivable that the LP34-binding site on K6 may undergo a conformational change in K6/K16 complexes to unmask the epitope. This does not occur in the basal layer of OSF epithelium as K16 is absent, whereas in the suprabasal layers both K6 and K16 are expressed, inducing LP34 binding. The conformation-specific epitope phenomenon has been reported for a number of keratin antibodies (28–32) and requires further investigation as LP34 is widely used in diagnostic pathology. Nevertheless, LHK6B staining in the basal layer of oral epithelium appears to be a characteristic feature of OSF in this study. Furthermore, as the basal layer predominantly contains proliferating cells and the suprabasal cells are derived from basal keratinocytes, anomalous expression of K6 in the basal layer is likely to influence subsequent differentiation-related cellular features such as keratinization and epithelial atrophy. Interestingly, this K6 expression pattern is also exhibited by normal oral keratinocytes induced to differentiate in an organotypical culture (27).

Reduced expression of K19 and K17 in basal keratinocytes of the OSF epithelium

The sporadic K19 expression in the basal layer of normal non-keratinized oral epithelia is consistent with that reported in the literature. Decreased K19 expression in OSF may be symptomatic of the keratinized state of the tissue as K19 expression is known to be incompatible with keratinization in normal oral epithelia (33, 34).

K17 has been described as both an oral epithelial basal cell marker (35) and a suprabasal hyperproliferation

Table 3 Loss of basal K17 expression in a subset of moderate-to-severe oral submucous fibrosis (OSF) cases

	K17 staining		*OSF histological grading*		
Sample	*Basal*	*Suprabasal*	*Early*	*Intermediate*	*Advanced*
OSF 1	−	+			+
OSF 11	−	+			+
OSF 28	−	+ +			+
OSF 33[a]	−	+ +		+	
OSF 4	+ +	−		+	
OSF 21	+ + +	−		+	
OSF 23	+ +	−	+		
OSF 35	+ +	−		+	
OSF 41	+ + +	−		+	

[a]Evidence of dysplastic change in the epithelium.

marker similar to K16 in epidermal wound healing (26, 36, 37). Our data are consistent with the former as expression of K17 is predominantly in the basal layer of normal epithelium with sparse suprabasal expression in 50% of samples. Table 3 shows four of 28 OSF samples in which there was no basal expression of K17 and 3 of these were amongst the most advanced OSF cases, whilst the other was classified as intermediate with evidence of dysplasia. Conversely, five of 28 samples expressed basal but no suprabasal K17 and none was advanced case. Loss of basal K17 appears to be a feature of certain advanced OSF pathologies in a particular cytokine environment such as the low level of interferon-γ found in some OSF lesions (38). This premise is consistent with the fact that interferon-γ is known to induce K17 in keratinocytes (39, 40). Perhaps K17 expression is more sensitive to interferon-γ than K15 because K15 is also induced by interferon-γ (41) but it remains uninfluenced by OSF pathology (Fig. 2i,j). We also note that, despite the small number of samples, four of 28 OSF tissues (14%) showing loss of K17 in the basal layer, as well as being biopsied from some of the most advanced lesions, is the same number that would be projected to progress to oral squamous cell carcinoma.

Induction of K17 in the suprabasal layers of OSF epithelium correlates with disease severity

Although K17 expression in the basal layer was absent in a subset of the most severe cases, overall there was no correlation between the basal K17 expression and disease severity. However, expression in the suprabasal layers correlated with histological grading of OSF severity whereby the most significant K17 increase occurred in the most advanced cases (Fig. 6). None of the other keratins influenced in OSF showed any significant correlation with the disease severity. These observations suggest that perturbation in K17 expression is perhaps a primary outcome of OSF pathology and the expression of other keratins is influenced by factors in addition to the disease pathology such as keratinization or dysplasia.

Keratinization in OSF epithelium has a molecular signature distinct from that in pre-cancerous dysplasia

The commonest pre-cancerous dysplasias are keratinized; however, hyperkeratinization does not always lead to dysplasia. Virtually all of our OSF samples showed evidence of keratinization but dysplastic changes were present only in seven samples. There are several lines of evidence to suggest that OSF keratinization may be different from that seen in hyperkeratotic lesions or dysplasia. In pre-cancerous dysplasia K16 has been reported to be induced (42) whilst we did not observe any change in the expression of this protein in OSF epithelium. The complete absence of K19 in OSF epithelium observed in this study is in contrast to its reported induction in pre-cancerous dysplasia (33). Furthermore, in hyperkeratotic lesions and in pre-cancerous dysplasia we have reported an upregulation of keratin K2 (11) when in OSF epithelium we did not observe K2 expression. Taking together these observations strongly suggest that keratinization in OSF may have a molecular signature clearly distinct from that observed in hyperkeratotic lesions and dysplasia. These data provide a foundation for future molecular analysis using expression microarrays.

In conclusion, we have provided definitive data to show that OSF results in an altered keratinocyte phenotype. We have shown an induction of keratinization-specific keratins K1 and K10 in the suprabasal layers of OSF epithelium. In the basal layer we found increased K6 and decreased K19 expression. K17 expression in the basal layer was downregulated only in a subset of the most severe cases, whereas in the suprabasal layers K17 expression increased with disease severity. This altered keratinocyte phenotype in OSF, indicates a potential mechanism for the chronic connective tissue pathology via perturbed epithelial mesenchymal interactions as well as the malignant conversion to oral squamous cell carcinoma.

Supplementary material

The following supplementary material is available for this article online:

Table S1: Keratin immunostaining of OSF and normal epithelial samples: correlation between visual assessment and pixel analysis

This material is available as part of the online article from http://www.blackwell-synergy.com

Please note: Blackwell Publishing are not responsible for the content or functionality of any supplementary materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

References

1. Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S. Oral submucous fibrosis: review on aetiology and pathogenesis. *Oral Oncol* 2006; **42**: 561–8.
2. Petersen PE. Strengthening the prevention of oral cancer: the WHO perspective. *Community Dent Oral Epidemiol* 2005; **33**: 397–9.
3. Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. *IARC Monogr Eval Carcinog Risks Hum* 2004; **85**: 1–334.
4. Utsunomiya H, Tilakaratne WM, Oshiro K, et al. Extracellular matrix remodeling in oral submucous fibrosis: its stage-specific modes revealed by immunohistochemistry and in situ hybridization. *J Oral Pathol Med* 2005; **34**: 498–507.
5. Kuo MY, Chen HM, Hahn LJ, Hsieh CC, Chiang CP. Collagen biosynthesis in human oral submucous fibrosis fibroblast cultures. *J Dent Res* 1995; **74**: 1783–8.
6. Pindborg JJ, Murti PR, Bhonsle RB, Gupta PC, Daftary DK, Mehta FS. Oral submucous fibrosis as a precancerous condition. *Scand J Dent Res* 1984; **92**: 224–9.
7. Canniff JP, Harvey W, Harris M. Oral submucous fibrosis: its pathogenesis and management. *Br Dent J* 1986; **160**: 429–34.
8. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression

in normal epithelia, tumors and cultured cells. *Cell* 1982; **31**: 11–24.
9. Schweizer J, Bowden PE, Coulombe PA, et al. New consensus nomenclature for mammalian keratins. *J Cell Biol* 2006; **174**: 169–74.
10. Waseem A, Dogan B, Tidman N, et al. Keratin 15 expression in stratified epithelia: downregulation in activated keratinocytes. *J Invest Dermatol* 1999; **112**: 362–9.
11. Bloor BK, Tidman N, Leigh IM, et al. Expression of keratin K2e in cutaneous and oral lesions: association with keratinocyte activation, proliferation, and keratinization. *Am J Pathol* 2003; **162**: 963–75.
12. Pindborg JJ, Sirsat SM. Oral submucous fibrosis. *Oral Surg Oral Med Oral Pathol* 1966; **22**: 764–79.
13. Elie N, Plancoulaine B, Signolle JP, Herlin P. A simple way of quantifying immunostained cell nuclei on the whole histologic section. *Cytometry A* 2003; **56**: 37–45.
14. Mackenzie IC. The role of epithelial-mesenchymal interactions in epithelial migration and differentiation. *J Periodontal Res* 1984; **19**: 656–60.
15. Mueller MM, Fusenig NE. Tumor-stroma interactions directing phenotype and progression of epithelial skin tumor cells. *Differentiation* 2002; **70**: 486–97.
16. Chinnathambi S, Tomanek-Chalkley A, Ludwig N, et al. Recapitulation of oral mucosal tissues in long-term organotypic culture. *Anat Rec A Discov Mol Cell Evol Biol* 2003; **270**: 162–74.
17. Mackenzie IC, Hill MW. Maintenance of regionally specific patterns of cell proliferation and differentiation in transplanted skin and oral mucosa. *Cell Tissue Res* 1981; **219**: 597–607.
18. Andriessen MP, Niessen FB, Van de Kerkhof PC, Schalkwijk J. Hypertrophic scarring is associated with epidermal abnormalities: an immunohistochemical study. *J Pathol* 1998; **186**: 192–200.
19. Machesney M, Tidman N, Waseem A, Kirby L, Leigh I. Activated keratinocytes in the epidermis of hypertrophic scars. *Am J Pathol* 1998; **152**: 1133–41.
20. Bloor BK, Seddon SV, Morgan PR. Gene expression of differentiation-specific keratins (K4, K13, K1 and K10) in oral non-dysplastic keratoses and lichen planus. *J Oral Pathol Med* 2000; **29**: 376–84.
21. Rajalalitha P, Vali S. Molecular pathogenesis of oral submucous fibrosis – a collagen metabolic disorder. *J Oral Pathol Med* 2005; **34**: 321–8.
22. Moore RJ, Owens DM, Stamp G, et al. Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. *Nat Med* 1999; **5**: 828–31.
23. Lane EB, Alexander CM. Use of keratin antibodies in tumor diagnosis. *Semin Cancer Biol* 1990; **1**: 165–79.
24. Ogden GR, Lunny D, Lane EB. Keratin antigen retrieval in oral mucosal biopsies using microwave processing. *Histochem J* 1996; **28**: 875–82.
25. Ranganathan K, Kavitha R, Sawant SS, Vaidya MM. Cytokeratin expression in oral submucous fibrosis–an immunohistochemical study. *J Oral Pathol Med* 2006; **35**: 25–32.
26. Van der Velden LA, Manni JJ, Ramaekers FC, Kuijpers W. Expression of intermediate filament proteins in benign lesions of the oral mucosa. *Eur Arch Otorhinolaryngol* 1999; **256**: 514–9.
27. Hansson A, Bloor BK, Haig Y, Morgan PR, Ekstrand J, Grafstrom RC. Expression of keratins in normal, immortalized and malignant oral epithelia in organotypic culture. *Oral Oncol* 2001; **37**: 419–30.
28. Waseem A, Lane EB, Harrison D, Waseem N. A keratin antibody recognizing a heterotypic complex: epitope mapping to complementary locations on both components of the complex. *Exp Cell Res* 1996; **223**: 203–14.
29. Waseem A, Karsten U, Leigh IM, Purkis P, Waseem NH, Lane EB. Conformational changes in the rod domain of human keratin 8 following heterotypic association with keratin 18 and its implication for filament stability. *Biochemistry* 2004; **43**: 1283–95.
30. Leers MP, Kolgen W, Bjorklund V, et al. Immunocytochemical detection and mapping of a cytokeratin 18 neo-epitope exposed during early apoptosis. *J Pathol* 1999; **187**: 567–72.
31. Ditzel HJ, Strik MC, Larsen MK, et al. Cancer-associated cleavage of cytokeratin 8/18 heterotypic complexes exposes a neoepitope in human adenocarcinomas. *J Biol Chem* 2002; **277**: 21712–22.
32. Waseem A, White K, Waseem NH. Identification of a novel keratin epitope: evidence for association between non-helical sub-domains L12 during filament assembly. *Int J Biochem Cell Biol* 1997; **29**: 971–83.
33. Su L, Morgan PR, Lane EB. Keratin 14 and 19 expression in normal, dysplastic and malignant oral epithelia. A study using in situ hybridization and immunohistochemistry. *J Oral Pathol Med* 1996; **25**: 293–301.
34. Lindberg K, Rheinwald JG. Suprabasal 40 kD keratin (K19) expression as an immunohistologic marker of premalignancy in oral epithelium. *Am J Pathol* 1989; **134**: 89–98.
35. Su L, Morgan PR, Lane EB. Protein and mRNA expression of simple epithelial keratins in normal, dysplastic, and malignant oral epithelia. *Am J Pathol* 1994; **145**: 1349–57.
36. Bowden PE. Keratins and other epidermal proteins. In: Priestley GC, Ed. *Molecular aspects of dermatology*. London: John Wiley & Sons Ltd, 1993; 19–54.
37. Tomic-canic M, Komine M, Freedberg IM, Blumenberg M. Epidermal signal transduction and transcription factor activation in activated keratinocytes. *J Dermatol Sci* 1998; **17**: 167–81.
38. Haque MF, Meghji S, Khitab U, Harris M. Oral submucous fibrosis patients have altered levels of cytokine production. *J Oral Pathol Med* 2000; **29**: 123–8.
39. Jiang CK, Flanagan S, Ohtsuki M, Shuai K, Freedberg IM, Blumenberg M. Disease-activated transcription factor: allergic reactions in human skin cause nuclear translocation of Stat-91 and induce synthesis of keratin K17. *Mol Cell Biol* 1994; **14**: 4759–69.
40. Bonnekoh B, Huerkamp C, Wevers A, et al. Up-regulation of keratin 17 expression in human hacat keratinocytes by interferon-gamma. *J Invest Dermatol* 1995; **104**: 58–61.
41. Radoja N, Stojadinovic O, Waseem A, et al. Thyroid hormones and gamma interferon specifically increase K15 keratin gene transcription. *Mol Cell Biol* 2004; **24**: 3168–79.
42. Ogden GR, Chisholm DM, Leigh IM, Lane EB. Cytokeratin profiles in dyskeratosis congenita: an immunocytochemical investigation of lingual hyperkeratosis. *J Oral Pathol Med* 1992; **21**: 353–7.

Acknowledgements

The authors would like to thank Professor Ian C. Mackenzie for valuable comments on the manuscript. We also thank Mr Mohammad Ikram for providing us almost unlimited supplies of ready-to-use monoclonal antibody supernatants for this study.

British Journal of Cancer (2009) 101, S68 – S72

www.bjcancer.com

Full Paper

Oral cancer screening in the Bangladeshi community of Tower Hamlets: a social model

H Nunn*[,1], A Lalli[2], F Fortune[2] and R Croucher[2]

[1]Health Information Department, Cancer Research UK, 61 Lincoln's Inn Fields, London WC2A 3PX, UK; [2]Centre for Clinical and Diagnostic Oral Sciences, Institute of Dentistry, Barts and the London School of Medicine and Dentistry, Queen Mary, University of London, Turner Street, London, UK

BACKGROUND: UK oral cancer incidence has risen by 22% in the last 10 years. Oral cancer is often detected at a late stage when treatment is debilitating and the chances of survival are poor. Certain black and minority ethnic groups are at elevated risk of oral cancer due to the prevalence of risk factor behaviours. We describe the background to, the development of and outcomes of an oral cancer screening activity appropriate to the needs of members of a disadvantaged community at high risk of oral cancer, carried out between 2006 and 2008 in Tower Hamlets, East London.

METHODS: In all, 1320 people participated during 34 days of screening, divided into two phases (Phase I (2006/2007): $n = 485$, Phase II (2008): $n = 835$). Modifications to the delivery process were implemented for Phase II in an attempt to recruit more high-risk individuals and to improve screening specificity.

RESULTS: In total, 75 people were urgently referred for further investigation (Phase I: $n = 20$, Phase II: $n = 55$). Nine were diagnosed with dysplastic lesions (Phase I: $n = 3$, Phase II: $n = 6$) and a further eight showed potentially malignant disorders without dysplasia (Phase I: $n = 1$, Phase II: $n = 7$). Screening participants with low levels of completed education (OR: 6.94, 95% CI: 1.66, 28.98) and who chewed paan with tobacco (OR: 8.01, 95% CI: 3.54, 18.08) were more likely to be referred for further investigation.

CONCLUSION: The project offers insights for the further development of oral cancer screening interventions for disadvantaged communities.

British Journal of Cancer (2009) **101,** S68 – S72. doi:10.1038/sj.bjc.6605394 www.bjcancer.com

Keywords: oral cancer; screening; high risk; disadvantaged communities; Bangladeshi

Oral cancer is defined as cancers of the lip, tongue, oral cavity, oropharynx, hypopharynx and piriform sinus. The majority of tumours are squamous cell carcinomas, with the most common sites being the oral cavity (31%) and the tongue (29%). The least common site for oral cancer is the lip, accounting for only 6% of cases (Office for National Statistics, 1996 – 2005). UK oral cancer incidence rates have increased by 22% in the last 10 years (Office for National Statistics, 1996 – 2005). In 2005, 4900 people were diagnosed with oral cancer in this country (Office for National Statistics, 1996 – 2005). There are inequalities in oral cancer incidence and survival, with rates varying according to level of deprivation, geographical location and ethnicity (Office for National Statistics, 1996 – 2005; NCIN, 2009). The available data suggest that risk of oral cancer is higher among older Asian females than in females from the general population, although this is not the case for males (NCIN, 2009).

The average 5-year survival rate for cancer of the oral cavity is around 50% (Office for National Statistics, 1996 – 2005). This is because the majority of cases are diagnosed at a late stage, when treatment is debilitating and the chances of survival are poor (Murphy *et al*, 2007). Oral cancer survival varies considerably by stage at diagnosis and by site (Office for National Statistics, 1996 – 2005). Treatment for early stage oral cancer or oral dysplasia, tends to be considerably less invasive and debilitating (Lodi *et al*, 2006; van der Waal, 2009).

*Correspondence: H Nunn; E-mail: hazel.nunn@cancer.org.uk

The Bangladeshi community in Tower Hamlets

Oral cancer is undoubtedly an important public health issue, but as it is a relatively rare disease in the United Kingdom, targeted population strategies are likely to be most cost effective. From 2005 to 2008, Cancer Research UK ran an oral cancer project targeted at the Bangladeshi community in Tower Hamlets, East London, funded largely by the Chief Dental Officer, Department of Health.

Tower Hamlets is the third most deprived borough in England (Office for National Statistics, 2001). One third of the borough's population is Bangladeshi, totalling nearly 66 000. This is the largest Bangladeshi community outside of Bangladesh (Office for National Statistics, 2001). Conducting the project in this borough offered the opportunity to closely target a large community at high risk of oral cancer residing in a relatively small geographical area in a deprived part of London.

The Bangladeshi community exhibits a high prevalence of a number of oral cancer risk factors, including smoking, chewing tobacco and chewing areca nut but excluding alcohol consumption when compared with the general adult population (Croucher *et al*, 2003; Health Survey for England, 2004). Forty per cent of Bangladeshi men report being current smokers, compared to 24% of men from the general population. Although Bangladeshi

women are much less likely to report being current smokers compared to women from the general population (2% compared to 23%), national estimates of chewing tobacco or areca nut use range from 16% of Bangladeshi women chewing paan with tobacco and 13% chewing paan without tobacco but with areca nut (Health Survey for England, 2004) to 48.5% of a sample of Bangladeshi women resident in Tower Hamlets chewing paan with tobacco (Croucher *et al*, 2003). As the Bangladeshi community of Tower Hamlets shows significantly different risk factor behaviours from the general UK population, highly targeted oral cancer screening is likely to be needed.

This paper aims to describe the development of an oral cancer screening activity appropriate to the needs of a disadvantaged community at high risk of oral cancer. Specifically, it will describe the delivery structure of the screening activity, explain the process followed and review and discuss some outcomes.

Oral screening and potentially malignant disorders

There is significant potential for early detection of oral cancer because the oral cavity and oropharynx are relatively accessible and amenable to examination without invasive procedures. The existence of potentially malignant disorders of the oral mucosa implies there should be significant potential for prevention of oral cancer through oral visual screening (Scully and Porter, 2000).

Potentially malignant disorders are defined as 'all clinical presentations that carry a risk of cancer' (Warnakulasuriya, 2007). They tend to be linked to tobacco use (Napier and Speight, 2008). The proportion of potentially malignant disorders that transform into frank oral cancers varies greatly, based on characteristics of the disorder and its site, the patient's age and gender (Napier and Speight, 2008), and the patient's behaviour (Warnakulasuriya *et al*, 2008; van der Waal, 2009). Overall malignant transformation rate for white patches (leukoplakias) is estimated at around 1% per year (van der Waal, 2009) while it is thought that between 75% and 90% of red patches (erythroplakias) will undergo malignant transformation (Scully and Porter, 2000).

Dysplastic change is reported to be the best predictor of future malignancy, and the more severe the dysplasia, the greater the likelihood of malignant transformation (Schepman *et al*, 1998). Estimates suggest that around 50% of patients with dysplasia may go on to develop oral squamous cell carcinoma (Napier and Speight, 2008).

The transformation rate of other potentially malignant disorders of the oral and oropharyngeal mucosa is harder to quantify, ranging from 0.13% to 2.2% for all potentially malignant disorders combined (Napier and Speight, 2008). Of specific relevance to an Asian population, where areca nut (betel quid) usage is prevalent, is oral sub-mucous fibrosis (OSF). This potentially malignant disorder has a reported malignant transformation rate of 14% (Tilakaratne *et al*, 2006).

A number of possible screening techniques have been proposed for oral cancer. The simplest involves visual examination of the oral mucosa (Kujan *et al*, 2006). A recent Cochrane review concluded that there was not enough evidence to determine whether oral screening by visual examination, or any other modality, in the general population could reduce mortality from oral cancer (Kujan *et al*, 2006), but an increasing number of studies suggest that oral screening could feasibly be carried out cost effectively as part of routine dental inspection in NHS general dental practice (Field *et al*, 1995; Lim *et al*, 2003; Speight *et al*, 2006). This may be of limited relevance to the Bangladeshi population of Tower Hamlets who are known to have poor attendance rates at General Dental Practitioners (Pearson *et al*, 1999).

Cuba is one of the only countries in the world to report a national oral cancer screening programme. This programme uses annual visual examination in dental practices (Fernandez Garrote *et al*, 1995). While there is some evidence that repeated screenings led to a reduced likelihood of advanced stage oral cancer (Sankaranarayanan *et al*, 2002), overall there has been limited evidence of a shift from advanced to earlier stage oral cancer following introduction of the programme (Fernandez Garrote *et al*, 1995).

MATERIALS AND METHODS

Developing a delivery structure

The following steps were followed in developing a protocol for screening activity:

1. *Establishment of a community advisory group* This comprised local stakeholders from the Primary Care Trust Smoking Cessation and Dental Access teams, Cancer Services, Oral Medicine and Oral Maxillofacial Surgery teams, community organisations, as well as practice nurses and patient and community representatives. The group offered guidance on the development and promotion of oral cancer screening activity among the Bangladeshi community and the development of accompanying oral cancer awareness literature.
2. *Formal qualitative research* was conducted with the target population, by means of focus group discussions and key informant interviews, to inform development of the oral cancer screening activity and accompanying literature.
3. *Identification of screening sites* in the locality to undertake screening activity where a mobile dental unit provided by the Primary Care Trust could be used.
4. *Screening criteria established* Oral cancer screening involved visual examination of the soft tissue of the oral cavity and oropharynx, and palpation of the neck using standardised criteria. The activity was conducted by two registered dental practitioners after refresher training to ensure compliance with guidance (National Institute for Health and Clinical Excellence, 2005).
5. *Development of referral pathways* Patients could be referred directly from the mobile dental unit, either to the Department of Oral Medicine, Barts and the London Dental Institute for further investigation, and/or to local stop tobacco services for cessation support.
6. *Determination of inclusion criteria* In addition to being Bangladeshi or British Bangladeshi and a resident of Tower Hamlets, inclusion criteria for screening included being aged at least 30 years and practising one or more of the following health-compromising behaviours: smoking tobacco, chewing tobacco, chewing paan (betel quid) without tobacco. Potential participants falling outside these inclusion criteria were not actively recruited, but if they persistently sought screening, whatever their age, gender, ethnicity or behaviour, they were not excluded.

The delivery process

Delivery of oral cancer screening in Tower Hamlets as part of this project was divided into two phases. *Phase I* involved 10 screening sessions, which took place in 2006 and 2007, and *Phase II* consisted of 24 screening sessions, which took place in 2008. Several refinements were made to the delivery process for Phase II.

In both phases, all screening sessions were held in the six wards within Tower Hamlets with the highest proportions of Bangladeshi residents based on ethnicity data from the 2001 census (Office for National Statistics, 2001). In Phase I, the 10 screening sessions were fairly evenly divided between these six wards, with exact locations of the mobile dental unit based on advice from the community advisory group. In Phase II, locations for conducting 24 oral cancer screening sessions were purposefully selected using ward ethnicity data from the 2001 census (Office for National Statistics, 2001), with the number of sessions conducted in each ward proportional to the size of the Bangladeshi population in that ward, with a higher number of screening sessions held in wards

with the highest proportions of Bangladeshi residents. The exact location within each ward was again based on advice from our community advisory group.

Screening was undertaken using a mobile dental unit – a specifically adapted motor vehicle, housing a dental clinic with conventional reclining dental chair and telescopic high-intensity lighting. This provided a clean, safe and confidential environment for examination of the illuminated oral mucosa in the supine position with the availability of all the instruments and amenities that would be present in a permanent dental clinic. Use of the mobile dental unit addressed all as the issues arising from pre-trial attempts at mucosal examination using hand-held portable equipment, including inconsistent lighting intensity, confidentiality and modesty concerns when examining in busy areas, difficulty gaining adequate oral mucosal access without the patient in the supine position, concerns about the examiners' posture and lack of appropriate cross-infection control.

Ensuring the cultural acceptability of oral cancer screening activity was addressed by the use of bi-lingual (English/Sylheti) advocates. Again, the procedure was modified between Phase I and Phase II. In Phase I, the main roles of bilingual advocates were to provide language support during and after screening and to facilitate the referral process by following up referred patients individually. In Phase II, the advocates had a more integral role in the delivery process, continuing to provide language and other support during and after screening, recruiting participants to tobacco cessation, facilitating the referral process and collecting evaluation data and promoting oral cancer screening activity. As well as encouraging passing pedestrians to take part, this promotion of oral cancer screening involved placing oral cancer awareness and advertising health education material in areas where, from their local knowledge, the advocates knew that older Bangladeshis lived, or visited, such as community centres, shops and sheltered accommodation. In addition, at the referral stage, the advocates facilitated the distribution of referral letters, followed up the letters with telephone calls to ensure the letters had been received and understood, and offered to accompany patients to their referral appointment if required. This process ensured as many patients as possible complied with referral.

Final modifications to the delivery process for Phase II concerned selection, training and calibration of the dentists conducting screening. For Phase I, dentists with varying levels of experience in the diagnosis and management of oral cancer were recruited. They were standardised by attendance at a pre-screening refresher session where images of oral mucosal lesions were examined in relation to the NICE guidelines on the referral of suspected cancer (National Institute for Health and Clinical Excellence, 2005). A more robust standardisation and calibration process was developed for Phase II based on an updated protocol (Ikeda *et al*, 1995; Barnes *et al*, 2005). Just two dentists were recruited for Phase II, both with extensive clinical experience in diagnosing oral mucosal pathology at secondary referral centres.

Assessing outcomes

The following outcomes of the activity are reported:

- numbers (Phases I and II) and demographic details (Phase II only) of screening participants and details of recruitment to local tobacco cessation services (Phase II only)
- numbers referred to secondary care services (Phases I and II)
- compliance with referral (Phases I and II)
- clinical outcomes and diagnoses (Phases I and II)
- socio-demographic and behavioural predictors of referral (Phase II only) using a retrospective case–control design, whereby participants referred were matched with multiple controls to increase study power
- financial considerations

During Phase II, a questionnaire was used to identify potential screening participants. Those identified as meeting the inclusion criteria were then asked to take part in a second interview. Information about age, gender, years of completed education, economic activity, self-reported health, oral cancer awareness and tobacco consumption was collected. The core content of the interview was provided by using validated questions taken from existing inventories (Humphris *et al*, 1999; Health Survey for England, 2004).

Data collection were approved by the local research ethics committee and analysed using STATA version 10. Participants gave their written consent to take part in the interview.

RESULTS

Characteristics of screened population and recruitment to tobacco cessation

Between 2006 and 2008, a total of 34 screening sessions were undertaken in Tower Hamlets with 1320 individuals screened (485 in Phase I and 835 in Phase II). On average, 39 people were screened per session (range 19–82). Each session was $\sim$7 h in duration, undertaken between 0930 and 1630 with flexible break times dependent on the flow of patients.

The mean age of the screened population was 42.3 years (SD 15.9 years). As shown in Table 1, 45% of patients screened in Phase II were female, 84% were from the Bangladeshi community and 58% were tobacco or areca nut users. In Phase II, a total of 202 screening participants were recruited to tobacco cessation (i.e. 42% of participants who were tobacco users). Data collection in Phase I were incomplete.

Referral, compliance and clinical outcomes

Of the 1320 individuals screened, 75 were referred for Oral Medicine consultation at Barts and The London Dental Institute. Table 2 shows the outcomes of these referrals. Despite attempts to ensure as many patients as possible complied with referral, 20 (27%) failed to attend either their initial hospital appointment or subsequent follow-up appointments such that a definitive diagnosis of their condition could not be made. Six out of 14 patients failed to comply with referral in Phase I (30%), while 14 out of 55 failed to comply in Phase II (25%). In all, 55 patients did attend after referral, and further investigations were instigated as appropriate to generate the diagnoses shown in Table 2.

None of those referred presented with frank oral cancer but 17 (31%) were diagnosed with potentially malignant disorders as shown in Table 2. Of these, nine had evidence of dysplasia (two moderate/severe) and five had OSF. The remaining 38 patients had benign lesions, of which the most common was keratosis ($n=31$).

Of the 55 patients attending the secondary referral service at Barts and the London, 35 (64%) remain under regular review including all those with potentially malignant disorders. These individuals exhibit a number of risk factors for the development

Table 1 Details of screening participants in Phase II

	Phase II (2008)
Number of screening sessions	24
Number of individuals screened	835
Number of females screened (%)	375 (45%)
Number of Bangladeshis screened (%)	762 (91%)
Number of tobacco/areca nut users screened (%)	485 (58%)
Number of recruits to tobacco cessation	202

of oral cancer and their pre-existing mucosal lesions indicate a significant risk of further lesions developing. Only 20 patients presenting with confirmed benign lesions and minimal risk factors, who had access to appropriate follow-up within primary dental care services, were able to be discharged from further review in the Department of Oral Medicine. In Phase I, 7 out of 14 (50%) of compliant referred patients remain under review, whereas in Phase II, 28 out of 41 compliant patients (68%) remain under review.

Socio-demographic and behavioural predictors of referral

Data were available for 49 of the 55 patients referred in Phase II. They were matched with 344 non-referred controls. Features of the cases and controls are reported in Table 3. Screening participants with low levels of completed education (OR: 6.94, 95% CI: 1.66, 28.98) and who chewed paan with tobacco (OR: 8.01, 95% CI: 3.54, 18.08) were more likely to be referred to the secondary care service. For other characteristics – economic activity, general health, smoking cigarettes, bidi and chewing paan without tobacco – there were no statistically significant differences in the screened population compared with the referred population.

Table 2 Definitive diagnosis by secondary referral centre

Clinical/histological diagnosis	Number of patients (of those who attended oral medicine clinic)		
	Phase I	Phase II	Total
Potentially malignant disorders			
Dysplasia	3	6	9
Oral sub-mucous fibrosis	1	4	5
Lichen planus	0	3	3
Total potentially malignant disorders	4	13	17
Benign lesions			
Keratosis	9	22	31
Candidiasis	0	1	1
Fibro-epithelial polyp	0	1	1
Physiological pigmentation	1	2	3
Haemangioma	0	2	2
Total benign lesions	10	28	38
Total	14	41	55

Financial considerations

The 34 screening sessions in Tower Hamlets cost ~£32 000 including all associated direct costs such as staff and hire of fully equipped mobile dental units but not including consultant time and hospital facilities for follow-up appointments. As 1320 people were screened during this period, the cost per screen was ~£24, which is approximately half the figure for the NHS breast-screening programme that stands at £45.50 per screen.

DISCUSSION

This project has shown the feasibility of conducting oral cancer screening in a deprived borough in East London, using a mobile dental clinic with dental practitioners undertaking the screening, supported by ethnically matched advocates from the local community. In line with the aims of the project, over 90% of the screening attendees in Phase II were from the local Bangladeshi community. This project also confirms the importance of providing oral health services in community settings (Croucher and Sohanpal, 2006).

Of the 1320 people screened, 75 (5.7%) were referred to secondary care for further investigation. This is in line with previous UK oral cancer screening initiatives (Downer *et al*, 2006) and is almost identical to that observed in a large screening trial in Kerala, India (Sankaranarayanan *et al*, 2005). Those referred were more likely to be paan with tobacco chewers, confirming previous findings (Pearson *et al*, 1999; Jayalekshmi *et al*, 2009), and to have limited years of completed education.

Only 55 (73%) of the 75 referred to secondary care attended their appointment despite considerable efforts being made both by the secondary referral centre and the local advocates. This is clearly a concern. However, this attendance rate is comparable with that in other studies (Downer *et al*, 2006) and is somewhat higher than the 63% reported for the Kerala study (Sankaranarayanan *et al*, 2005). It is also worthy of note that in the national bowel cancer screening programme ~20% of those with a positive faecal occult blood test do not attend for colonoscopy.

Reasons for non-attendance at secondary care were investigated by telephoning patients who initially failed to attend, but subsequently did so. Common reasons for initial non-attendance included language barriers, non-receipt of referral letter and a perceived difficulty in attending hospital. Among the 20 patients who never attended secondary care, it has been established that two had returned to Bangladesh but it was not possible to

Table 3 Predictors of referral

Variables	Univariate analysis: OR (95% CI)	*P*-value	Multivariate analysis: OR (95% CI)	*P*-value
Years in education				
19+	1.00		1.00	
15–18	2.81 (0.97–8.13)	0.04	1.98 (0.61–6.41)	0.25
Under 14	1.64 (0.57–4.69)	0.34	2.02 (0.62–6.57)	0.24
None	3.6(10.20–41.30)	0.01	6.94 (1.66–28.98)	0.01
Economically active	0.70 (0.29–1.68)	0.42	0.32 (0.09–1.15)	0.08
Self-reported health				
Good-excellent	1.11 (0.57–2.17)	0.73	1.60 (0.71–3.58)	0.25
Self-reported oral health				
Good-excellent	0.87 (0.39–1.94)	0.74	0.77 (0.33–1.78)	0.55
Any oral pain	0.98 (0.51–1.91)	0.97	0.86 (0.61–1.23)	0.43
Smoke cigarettes	0.81 (0.32–2.00)	0.65	0.64 (0.22–1.88)	0.42
Smoke bidi	1.58 (0.14–18.06)	0.70	1.84 (0.14–23.04)	0.63
Chew paan with tobacco	5.69 (2.66–12.18)	0.00	8.01 (3.54–18.08)	0.00
Chew paan without tobacco	0.56 (0.26–1.17)	0.12	0.73 (0.29–1.81)	0.50
OC awareness: 5+ correct	1.02 (0.52–2.04)	0.93	1.02 (0.53–2.45)	0.73

Results of univariate and multivariate conditional logistic analysis.

determine whether this was as a direct result of the screening outcome and associated psychological burden.

Among the 55 patients who attended secondary care, 17 (31%) were found to have potentially malignant disorders. Benign oral mucosal hyperkeratosis was by far the most common lesion, occurring in 31 (56%) of the 55 individuals. This is consistent with a paan chewing habit, where the rough fibres of the betel quid cause frictional damage to the epithelial surface of the mucosa (Lalli *et al*, 2008). In total, 35 (64%) of the 55 attendees at secondary care remain under follow-up because of their mucosal condition and associated risk factors. These can be considered as positive referrals.

One of the unique features of this project was the linkage between oral cancer and the tobacco cessation programme. In Phase II of the project, over 40% of tobacco users attending screening (202 of 485) were recruited to tobacco cessation.

Future developments

This project has shown the feasibility and acceptability of oral cancer screening using a mobile dental unit among the Bangladeshi community of Tower Hamlets. It would now be valuable to test this approach in other high-risk communities.

Further investigation into the reasons for non-compliance with referral to secondary care is needed. Innovative use of the mobile dental unit could eradicate delay in obtaining a definitive diagnosis from initial screening if suspicious lesions were biopsied in the field. A brush biopsy and immediate cytological analysis could be an appropriately quick and minimally invasive procedure to undertake on the mobile dental unit. There would, however, be significant cost implications.

ACKNOWLEDGEMENTS

We are grateful to colleagues who have assisted with this project and acknowledge the contribution of Shamsia Begum and Dipul Ghosh, Bangladeshi Stop Tobacco Project; Noha Seoudi, Stuart McEachen and John Buchanan, Barts and the London School of Medicine and Dentistry; Jessica Harris, Zoë Norridge and Sara Hiom, Cancer Research UK.

Conflict of interest
The authors declare no conflict of interest.

REFERENCES

Barnes L, Eveson J, Reichart P, Sidransky D (eds). (2005) *World Health Organisation Classification of Tumours. Pathology and Genetics of Tumours of the Head and Neck*. IARC Press: Lyon

Croucher R, Islam S, Jarvis M, Garrett M, Rahman R, Shajahan S, Howells G (2003) Oral tobacco cessation with UK resident Bangladeshi women: a community pilot investigation. *Health Educ Res* **18**(2)**:** 216 – 223

Croucher R, Sohanpal R (2006) Improving access to dental care in East London's ethnic minority groups: community-based, qualitative study. *Community Dent Health* **23**(2)**:** 95 – 100

Downer MC, Moles DR, Palmer S, Speight PM (2006) A systematic review of measures of effectiveness in screening for oral cancer and precancer. *Oral Oncol* **42**(6)**:** 551 – 560

Field EA, Morrison T, Darling AE, Parr TA, Zakrzewska JM (1995) Oral mucosal screening as an integral part of routine dental care. *Br Dental J* **179:** 262 – 266

Fernandez Garrote L, Sankaranarayanan R, Lence Anta JJ, Rodriguez Salvá A, Maxwell Parkin D (1995) An evaluation of the oral cancer control program in Cuba. *Epidemiology* **6:** 428 – 431

Health Survey for England (2004) Health of Ethnic Minorities. The Information Centre: London

Humphris GM, Duncalf M, Holt D, Field EA (1999) The experimental evaluation of an oral cancer information leaflet. *Oral Oncol* **35**(6)**:** 575 – 582

Ikeda N, Downer MC, Ozowa Y, Inoue C, Mizuno T, Kawai T (1995) Characteristics of participants and non-participants in annual mass screening for oral cancer in 60-year-old residents of Tokoname city, Japan. *Community Dent Health* **12**(2)**:** 83 – 88

Jayalekshmi PA, Gangadharan P, Akiba S, Nair RRK, Tsuji M, Rajan B (2009) Tobacco chewing and female oral cavity cancer risk in Karunagappally cohort, India. *Br J Cancer* **100:** 848 – 852

Kujan O, Glenny AM, Oliver RJ, Thakker N, Sloan P (2006) Screening programmes for the early detection and prevention of oral cancer. *Cochrane Database Sys Rev* **19:** 3

Lalli A, Tilakaratne WM, Ariyawardana A, Fitchett C, Leigh IM, Hagi-Pavli E, Cruchley AT, Parkinson EK, Teh MT, Fortune F, Waseem A (2008) An altered keratinocyte phenotype in oral sub-mucous fibrosis: correlation of keratin K17 expression with disease severity. *J Oral Pathol Med* **37**(4)**:** 211 – 220

Lim K, Moles DR, Downer DR, Speight PM (2003) Opportunistic screening for oral cancer and pre-cancer in general dental practice: results of a demonstration study. *Br Dent J* **194:** 497 – 502

Lodi G, Sardella A, Bez C, Demarosi F, Carrassi A (2006) Interventions for treating oral leukoplakia. *Cochrane Database Sys Rev* **18**(4)**:** CD001829

Murphy BA, Gilbert J, Cmelak A, Ridner SH (2007) Symptom control issues and supportive care of patients with head and neck cancers. *Clin Adv Hematol Oncol* **5**(10)**:** 807 – 822

Napier SS, Speight PM (2008) Natural history of potentially malignant oral lesions and conditions: an overview of the literature. *J Oral Pathol Med* **37:** 1 – 10

National Cancer Intelligence Network (2009) Cancer Incidence and Survival by Major Ethnic Group, England, 2002 – 2006

National Institute for Health and Clinical Excellence (2005) Referral guidelines for suspected cancer. NICE: London

Office for National Statistics (2001) *Census: Neighbourhood Ethnic Minority Statistics*. Stationary Office: London

Office for National Statistics (1996–2005). *Cancer Statistics Registrations* Registrations of Cancer Diagnosed in England. Stationary Office: London

Pearson N, Croucher R, Marcenes W, O'Farrell M (1999) Dental service use and the implications for oral cancer screening in a sample of Bangladeshi adult medical care users living in Tower Hamlets, UK. *Br Dent J* **186**(10)**:** 517 – 521

Sankaranarayanan R, Fernandez Garrote L, Lence Anta J, Pisani P, Rodriguez Salva A (2002) Visual inspection in oral cancer screening in Cuba: a case-control study. *Oral Oncol* **38**(2)**:** 131 – 136

Sankaranarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B, Rajan B (2005) Trivandrum Oral Cancer Screening Study Group. Effect of screening on oral cancer mortality in Kerala, India: a cluster-randomised controlled trial. *Lancet* **365**(9475)**:** 1927 – 1933

Scully C, Porter S (2000) ABC of oral health. Swellings and red, white and pigmented lesions. *BMJ* **321**(7255)**:** 225 – 228

Schepman KP, van der Meij EH, Smeele LE, van der Waal I (1998) Malignant transformation of oral leukoplakia: a follow-up study of a hospital-based population of 166 patients with oral leukoplakia from The Netherlands. *Oral Oncol* **34**(4)**:** 270 – 275

Speight PM, Palmer S, Moles DR, Downer MC, Smith DH, Henriksson M, Augustovski F (2006) The cost-effectiveness of screening for oral cancer in primary care. *Health Technol Assess* **10**(14)**:** 1 – 144, iii – iv

Tilakaratne WM, Klinikowski MF, Saku T, Peters TJ, Warnakulasuriya S (2006) Oral sub-mucous fibrosis: review on aetiology and pathogenesis. *Oral Oncol* **42:** 561 – 568

van der Waal I (2009) Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. *Oral Oncol* **45:** 317 – 323

Warnakulasuriya S (2007) Nomenclature and classification of potentially malignant disorders of the oral mucosa. *J Oral Pathol Med* **36**(10)**:** 575 – 580

Warnakulasuriya S, Reibel J, Bouquot J, Dabelsteen E (2008) Oral epithelial dysplasia classification systems: predictive value, utility, weaknesses and scope for improvement. *J Oral Pathol Med* **37**(3)**:** 127 – 133